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An empirical study on concentration-QTc model

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Abstract of the Dissertation

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QT interval is a measure of the time from the beginning of the Q wave to the end of the T wave in the heart's electrical cycle, it is often corrected to lessen its dependence to heart rate, and the corrected value is known as the QTc interval. Prolongation of the QTc interval is a primary biomarker for assessing proarrhythmic risk of drugs, thus it is routinely evaluated in new drug development. Concentration-QTc model with data from early phase single ascending dose (SAD) study is widely used to evaluate the drug-induced QTc prolongation, and the mixed effects model (MEM) is popular for this purpose. But some statistical issues in existing concentration-QTc model are rarely addressed, such as the limitation of the conventional sampling strategy in SAD study design, the baseline adjustment in crossover SAD study, and the violation of covariance matrix structure when applying the maximum likelihood (ML) method to analyzing the MEM. Several statistical issues in concentration-QTc model are addressed in this work.

First, multi-oscillator function chosen by some model fitting criteria, such as AIC, is widely used to account for circadian rhythm effect in concentration-QTc model. An evaluation of the limitations of the conventional SAD design sampling strategy to recover the true underlying oscillator function is given. Subsequently, we propose a modified sampling strategy to improve the ability to recover the true underlying function. The superiority of this new sampling strategy is examined and confirmed via simulation studies.

Secondly, in a crossover SAD study with period-specific pre-dose QTc baseline, the most efficient way to adjust pre-dose baseline in concentration-QTc model is unclear. We propose a novel conditional ΔQTc model by incorporating the pre-dose baseline and the pre-dose-averaged baseline as covariates in this model. We demonstrate the advantage of the proposed conditional ΔQTc model comparing to existing models, both analytically and through simulation studies.

Finally, given that the MEM with ML method is dominant in concentration-QTc model, we proposed several alternative modeling and analysis approaches. We first adopted the Bayesian method and compared which to the Frequentist ML method. Furthermore, since the ML method for MEM requires the correctly specified covariance matrix structure which is usually hard to verify, we examined the generalized estimating equation (GEE) and the generalized method of moments (GMM) as more robust alternatives and comparisons between these and the ML method are made.

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List of Abbreviations

AR(1): autoregressive with order 1

Cmax: time to reach the peak plasma concentration of a drug after administration

ECG: electrocardiogram

GEE: generalized estimating equation

GMM: generalized method of moments

IUT: intersection-union test

MEM: mixed effects model

ML: maximum likelihood

MSE: mean squared error

QT: QT interval, is a measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle

QTc: corrected QT interval

s.d.: standard deviation

SAD: single ascending dose

Tmax: the peak plasma concentration of a drug after administration

TQT: thorough QT

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Chapter 1 Introduction

Evaluation of QT/QTc interval prolongation via the "thorough QT/QTc" (TQT) study is essential in assessing cardiovascular safety in new drug application. Explicit guidelines for conducting the TQT study has been implemented in the International Conference of Harmonization (ICH) E14. However, it is widely accepted that TQT study has many disadvantages including low cost-effectiveness, requirement of large sample size, and high false positive rate. Since the plasma concentration of the drug and electrocardiogram (ECG) samples are also collected in phase 1 single ascending dose (SAD) clinical trials, using the concentration-QTc model as an alternative to the traditional TQT study in evaluation of QT/QTc prolongation is under active investigation. Various models are proposed by researchers, however several statistical issues are neglected in the current development of concentration-QTc models, such as (1) the limitation of conventional sampling strategy in SAD study design, (2) baseline adjustment of concentration-QTc model with data from crossover SAD study, and (3) violation of covariance matrix structure when using the maximum likelihood (ML) method for mixed effects model (MEM). In this dissertation, we endeavor to address these three issues. In the ensuing Chapter 1, we first provide a brief introduction to the QT/QTc interval in section 1.1. This is followed by a discussion on the deficits of TQT study in section 1.2, and a review of the current development of concentration-QTc models in Sections 1.3 and 1.4. Finally in Section 1.5, an outline of the dissertation is presented.

1.1 Introduction to QT/QTc interval

In a normal electrocardiogram (ECG), the QT interval is a measure of time between the beginning of the QRS complex to the end of the T-wave, it represents the

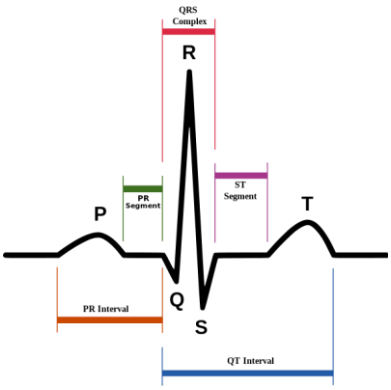

Figure 1.1 Schematic representation of normal ECG trace (https://en.wikipedia.org/wiki/Electrocardiography)

duration of ventricular depolarization and subsequent repolarization (Figure 1.1). A delay in cardiac repolarization may result in the development of ventricular tachyarrhythmias like torsade de pointes (TdP) which means "twisting of the spikes". Occurrence of TdP may cause dizziness, syncope and degenerate into ventricular fibrillation which is highly possible to lead to sudden death. Prolongation of the QT interval is regarded as an imperfect but widely-accepted biomarker in the assessment of the proarrhythmic risk of a therapeutic drug, and thus the evaluation of the QT prolongation is essential in the safety assessment of a new drug application.

RR interval is time measure from the onset of one QRS complex to the onset of the next QTS complex where QRS complex is an electrocardiogram corresponds to the depolarization of the right and left ventricles of the human heart, RR interval is the inverse of heart rate. The dependence of measured QT interval on RR interval is routinely corrected by different formulae, thus the corrected QT value, known as the QTc, is used in further analysis instead of the QT interval. A typical method for correcting the QT interval for RR interval is given by the formula:

$$QTc = QT/RR^{\alpha}.$$

The Bazzet's and the Fridericia's methods are the two most frequently used approaches to assign the α value with Bazett (1920) choosing $\alpha = -0.5$ while Fridericia (1920) using $\alpha = -1/3$. It is also recognized that α can be assigned to other values via the linear mixed effects model based on the observed data. Individual method (Malik and Camm 2001, Malik 2001, De sai et al. 2003) has also been proposed to allow subject-specific α if sufficiently large number of pretreatment QT interval measurements in each subject are available. The correction method is beyond the scope of this dissertation. In the following sections, QTc denotes the response of interest with an appropriate correction of QT values.

Other than the RR intervals, the QT/QTc interval are potentially affected by some subject-related factors, such as food intake, gender, obesity, age, alcoholism, etc. Between 16 to 23 milliseconds increase of the QT/QTc interval have been reported during the first 60 minutes after a meal (Nagy et al. 1997). Females also tend to have around 10 milliseconds longer QTc interval on average than males (Stramba-Badiale et al. 1997, Bonate et al. 2003). 10 kg increase in fat mass also links with around 5 milliseconds increase in QTc (Carella et al. 1996, El-Gamal et al. 1995). Moreover, QTc is subject to circadian rhythm effect (within-day variations). Figure

4 in Piotrovsky (2005) is an example of the circadian rhythm of QTc in healthy volunteers. A more detailed introduction to QTc circadian rhythm is given in Chapter 2.

1.2 Thorough QT/QTc (TQT) Study

1.2.1 Introduction to thorough QT (TQT) study

Since the 1980s, arising evidence reveals that some non-antiarrhythmic drugs significantly prolong QT/QTc interval and lead to cardiotoxic potential, even risk of sudden death (De Ponti et al. 2000, Malik and Camm 2001). Several drugs such as Terfenadine and Cisapride were withdrawn from the market due to such concerns, thus evaluation of the proarrhythmic potential of drugs before FDA approval is essential. The International Conference on Harmonization topic E14 (ICH E14) established clear guidelines in 2005. It requires a "thorough QT (TQT) study" as part of a new drug application.

A TQT study is a short-duration but intensive and expensive study to evaluate the potential risk of new drugs to prolong the QT/QTc interval. A TQT study is typically performed in either a crossover design or a parallel design.

Parallel design is suitable for drugs with long washout period, and it is conducted in healthy volunteers who are randomly assigned to one of the 4 treatment arms: placebo arm, therapeutic dose arm, supratherapeutic dose arm, and also a positive-control arm. For each subject, ECG measurements will be taken multiple times in the 24 hours following drug/placebo administration. In the day prior to drug administration, time-matched ECG measurements are taken as the baseline ECG evaluation. Typically, each arm consists of 40-80 subjects (Yan et al. 2010, Zhang 2008). An example of a TQT study in parallel design is shown in Figure 1.2.

Crossover design is usually suitable for drugs with short washout period, each subject will have placebo, therapeutic and supratherapeutic dose of tested drug and positive control in each of the 4 periods. At the beginning of each period, baseline ECG evaluation always consists of several ECG measurements right before the drug administration at each period.

The requirement of supratherapeutic dose arm in a TQT study is due to the proarrhythmic risk found to be significantly amplified by unexpected increases in drug concentration at approved doses resulting from drug metabolism. The aim of active-control is to assess the assay

sensitivity, usually a single dose of 400 mg moxifloxacin is used, at least one time point must show that a lower 1-sided 95% confidence interval greater than 5 ms for mean QTc baseline-adjusted change from placebo (Rohatagi et al. 2009) to establish assay sensitivity.

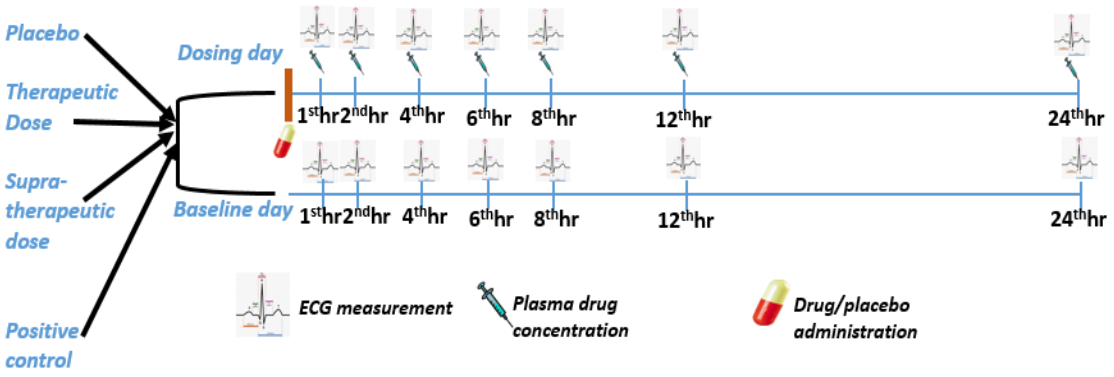

Figure 1.2 An example of a TQT study in parallel design

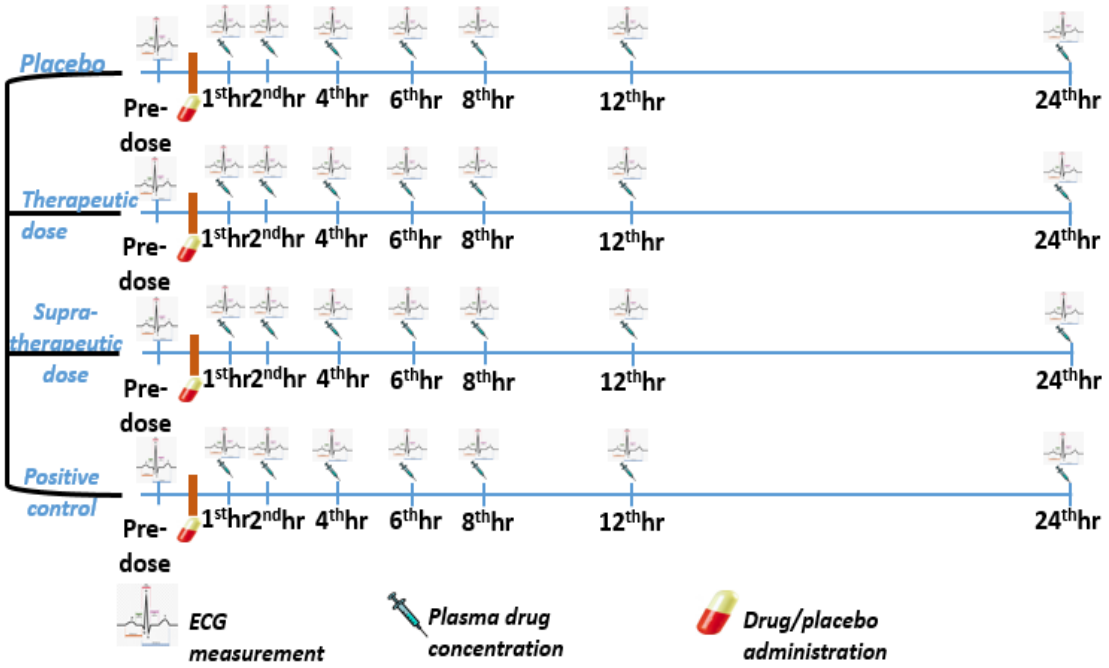

Figure 1.3 An example of a TQT study in crossover design

The primary endpoint for the TQT study is the time-matched and baseline-corrected QTc difference from placebo. According to ICH E14, a negative TQT study is defined as "one in which the upper bound of the 95% one-sided confidence interval for the largest time-matched mean effect of the drug on the QTc interval excludes 10 milliseconds (ms)". This definition is

chosen to provide a reasonable assurance that the mean effect of the study drug on the QT/QTc interval is not greater than 5 ms.

The common way to evaluate a TQT study is through an intersection-union test (IUT) as follows,

$$H_0: \bigcup_{s=1}^{T}\{\mu_1(t_s) - \mu_0(t_s) \geq 10\} \qquad VS \qquad H_a: \bigcap_{s=1}^{T}\{\mu_1(t_s) - \mu_0(t_s) < 10\},$$

where T is the number of ECG measurements at time points $t_1, t_2, \ldots, t_T$, $\mu_1(t_s)$ and $\mu_0(t_s)$ are the baseline corrected QTc values for drug and placebo arms respectively at *s*-th post-dose time point. Comparison between the therapeutic arm and the placebo arm, and between the supratherapeutic arm and the placebo arm are analyzed via IUT, respectively. Then 95% one-sided confidence intervals for $\mu_1(t_s) - \mu_0(t_s)$ at each time point were calculated. When the upper bound of the largest time-matched difference exceeds the threshold of 10 ms, the study is classified as "positive". The ICH E14 indicates that a positive study does not necessarily imply that the drug is proarrhythmic, however it has great influences on the evaluations of the drug carried out in the later stage of drug development, where additional ECG safety evaluation in subsequent clinical studies should be performed. In contrast, if the result of the "through QT/QTc study" is negative, requirements for ECG safety evaluations will be lessened in the later clinical trials. Similar IUT analysis is also applied between active control arm and placebo arm, as mentioned before, at least one time point has to have a lower 1-sided 95% confidence interval greater than 5 ms for the mean QTc baseline-adjusted change from placebo to establish assay sensitivity.

1.2.2 Drawbacks of TQT study

TQT study is known to be not cost-effective. Typically, a TQT study costs millions of dollars. If high-quality ECG data and appropriate experimental design can be implemented in other clinical trial, then the number of clinical trials needed would be reduced without losing the confidence in evaluating proarrhythmic risk. Phase 1 single ascending dose (SAD) study is a potential alternative since ECG evaluations and wide range of drug concentrations are available in phase 1 SAD studies.

Moreover, TQT study analyzed by IUT is known to have a high false-positive probability (Hutmacher et al. 2008) and may lead to wrong conclusion due to its binary "positive/negative"

decision rule. Russell et al. (2008) mentioned that when high variability in QT response is anticipated, a sample size of larger than 500 patients would be needed to achieve adequate power using the IUT to meet the ICH E14 definition of "negative study".

Last but not the least, due to tolerability concerns, a TQT study cannot be conducted in healthy volunteers in some clinical trials, such as those with certain oncology agents (Garnett et al. 2008). However evaluations of the QT prolongation risk of these drugs are also expected during the drug development process, and thus alternatives to the TQT study need to be considered to accomplish this goal.

1.3 Introduction to concentration-QTc model

Researchers have been actively investigating the role of concentration-QTc model in the evaluation of cardiovascular risk (Garnett et al. 2008, Darpo et al.2014, Russell et al. 2008, Rohatagi et al. 2009) by evaluating the relationship between drug concentration and QTc prolongation, instead of performing IUT on data from the TQT study. ICH E14 also mentioned the possibility of replacing the TQT study by a concentration-QTc model with data from early phase clinical studies in Section 2.2.5, which suggests "evaluating the relationship between concentration and QT/QTc effects or more intensively evaluating ECGs, based on data collected during early phase clinical studies". ICH E14 Q&A (2014) emphasized the value of concentration-QTc model and indicated that concentration-QTc model is "an important component of a totality of evidence assessment of the risk of QT prolongation", and it further suggested that concentration-QTc model can be built in early phase studies as part of the conventional QT study.

Comparing to conventional IUT analysis used in TQT study, concentration-QTc model with data from early phase clinical studies always contain a much wider range of dosage arms which exceeds the supratherapeutic dose, not only can it provide information of QTc prolongation on doses higher than the supratherapeutic dose, but it can also predict QTc prolongation at lower doses which are not included in the TQT study, and thus extensive knowledge of drug-induced QTc prolongation can be derived from concentration-QTc model. In addition, concentration-QTc model also allows a more thorough understanding of the variability inherent in the data.

In summary, if high-quality ECG measurements, elaborate experimental design and data analysis accomplished in early clinical studies are able to detect the QTc prolongation with high confidence, then it may obviate the need to conduct the TQT study entirely.

1.4 Current development of concentration-QTc model with data from crossover SAD design

Before the introduction to the current development of concentration-QTc models, crossover single ascending dose (SAD) design is introduced. Table 1.1 provides the experimental design of a typical 4-period crossover phase 1 SAD design. In each of the 4 periods, each subject is given either a placebo or a certain dosage of the tested drug. Usually a crossover design is suitable for drugs with short washout time, the time between adjacent periods are long enough to allow the drug to washout. In each period, each subject has several unevenly spaced time-matched measurements of drug concentration and ECG measurements in a 24-hour interval. Baseline ECG measurements are taken right before drug administration at each period. The sample size in a phase 1 SAD study is usually not very large (around 20).

Table 1.1 A typical design of a phase 1 crossover single ascending dose (SAD) study

PANEL	**Number of Subjects**	**Period 1**	**Period 2**	**Period 3**	**Period 4**
A	N=2	Placebo	DOSE 3	DOSE 5	DOSE 7
	N=2	DOSE 1	Placebo	DOSE 5	DOSE 7
	N=2	DOSE 1	DOSE 3	Placebo	DOSE 7
	N=2	DOSE 1	DOSE 3	DOSE 5	Placebo
B	N=2	Placebo	DOSE 4	DOSE 6	DOSE 8
	N=2	DOSE 2	Placebo	DOSE 6	DOSE 8
	N=2	DOSE 2	DOSE 4	Placebo	DOSE 8
	N=2	DOSE 2	DOSE 4	DOSE 6	Placebo

Table 1.2 Format of data from a phase 1 crossover single ascending dose (SAD) study for the k-th subject

Subject	Period	Time	QTc	Drug concentration
k	1	Baseline	$y_{k1,-1}$	0
		t_1	y_{k11}	C_{k11}
		t_2	y_{k12}	C_{k12}
		…	…	…
		t_S	y_{k1S}	C_{k1S}
	2	baseline	$y_{k2,-1}$	0
		t_1	y_{k21}	C_{k21}
		t_2	y_{k22}	C_{k22}
		…	…	…
		t_S	y_{k2S}	C_{k2S}
	.....	…	…	…
	J	baseline	$y_{kJ,-1}$	0
		t_1	y_{kJ1}	C_{kJ1}
		t_2	y_{kJ2}	C_{kJ2}
		…	…	…
		t_S	y_{kJS}	C_{kJS}

Here, we denote y_{kjs} as the QTc value for subject k ($k = 1,2, \dots, K$) at time t_s ($s = 1,2, \dots, T$) of period j ($j = 1,2, \dots, J$), and C_{kjs} denotes the drug concentration value for subject k at time t_s of period j. Furthermore, $s = -1$ indicates the pre-dose ECG measurement is taken at time t_{-1}. Let $\boldsymbol{y_k}$ and $\boldsymbol{C_k}$ be the $JT \times 1$ vector of the QTc value and concentration value for subject k, respectively. The QTc and drug concentration data for subject k is summarized in Table 1.2.

A key difference in different concentration-QTc models using data from crossover SAD studies with period-specific pre-dose QTc baseline lies in different ways to adjust the QTc baseline QTc. There are 3 types of concentration-QTc models: raw QTc model, ΔQTc model and ΔΔQTc model. The raw QTc (rQTc) model simply ignores the baseline information and model the post-dose QTc values directly, while the ΔQTc model takes the difference between the post-dose QTc value and pre-dose baseline QTc value and build model on the change from baseline QTc value (ΔQTc). The ΔΔQTc model calculates the ΔΔQTc by first calculating the ΔQTc values and then takes the differences of ΔQTc values between the treatment period and the placebo period, hence the ΔΔQTc model uses the change from placebo adjusted for baseline as

the response variable. This section gives a brief introduction to the three types of the concentration-QTc models, a comprehensive discussion on the model comparison is given in Chapter 3 and a novel conditional ΔQTc (cΔQTc) model is also proposed.

1.4.1 Raw QTc (rQTc) model

Piotrovsky (2005) gave a comprehensive introduction on how to build a concentration-QTc model based on the raw QTc value, including the use of multi-oscillator functions to model the circadian rhythm effect (time effect). Rohatagi et al. (2008) also presented how to formulate the rQTc model with multi-oscillator functions to model circadian rhythm effect. Huh and Hutmacher (2015) indicated that using time as a categorical variable in the model also provided high accuracy in estimation of the drug effect. The formulation of the rQTc is as follows.

$$\boldsymbol{y_k} = \beta * \boldsymbol{C_k} + \boldsymbol{T_k} + \boldsymbol{e_k}$$

Here, $\boldsymbol{y_k}$ and $\boldsymbol{C_k}$ are as defined above, $\beta * \boldsymbol{C_k}$ is the vector of drug induced QTc prolongation, while β is the slope of drug effect. Sometimes β is also allowed to vary between different subjects. Following convention, $\boldsymbol{e_k}$ is the error vector consists of the all random variation, including measurement error and other random effect for $\boldsymbol{y_k}$, and covariance structure of $\boldsymbol{e_k}$ is determined based on prior knowledge or the data we have.

The $JT \times 1$ vector $\boldsymbol{T_k}$ consists of the time effect T_{kjs} for subject k at time t_s in period j. It is used to model the circadian rhythm effect (time effect), which is usually modelled by incorporation of categorical time covariate in the model, or by using the multi-oscillator functions. The multi-oscillator function is formulated as follows.

$$T_{kjs} = QTc_0 * (1 + osc_{ks}),$$

$$osc_{ks} = amp24_k * \cos\left(\frac{2\pi*(t_s - acr24_k)}{24}\right) + amp12_k * \cos\left(\frac{2\pi*(t_s - acr12_k)}{12}\right) + amp6_k * \cos\left(\frac{2\pi*(t_s - acr6_k)}{6}\right).$$

Sometimes only one or two cosine functions (such as 24hr-osc+12hr-osc) are included in the model to account for the circadian rhythm effect. Typically, in concentration-QTc model with data from phase 1 SAD crossover study, only data from the placebo period are included in this part to pick up the choice of oscillator functions. The choice of oscillator functions among

various candidate functions is based upon a comparison of some model fitting criteria, such as AIC. Other covariates, such as gender, age, BMI (body mass index) etc. are also included in the model in an appropriate manner. After picking up the oscillator function, all the data will be pooled together to build the final model. A discussion on the use of oscillator function to model QTc circadian rhythm effect is provided in Chapter 2.

1.4.2 Change-From-Baseline QTc (ΔQTc) Model

Change-from-baseline QTc (ΔQTc) model is the most widely used method for concentration-QTc model which uses change-from baseline QTc as the response variable rather than the raw QTc.

Rohatgi et al. (2008) presented the calculation of ΔQTc and how to model drug effect with ΔQTc model. Azzam et al. (2015) also applied ΔQTc model on data from SAD design with categorical time effect. The definition of baseline for a crossover phase 1 SAD study is the pre-dose QTc measurement which is taken shortly prior to drug/placebo administration at each period, usually the average of 3 QTc baseline measurements is considered as the QTc baseline used in the analysis.

In the ΔQTc model, the circadian rhythm effect of QTc is also modeled by the oscillator function, or using time as a categorical variable. Here we denote the QTc change from baseline as $\boldsymbol{\Delta y_{kjs}}$ as follows:

$$\Delta y_{kjs} = y_{kjs} - y_{kj,-1}.$$

Here $y_{kj,-1}$ is the period-specific pre-dose QTc baseline measurement for subject k in period j. Let $\boldsymbol{\Delta y_k}$ be a $JT \times 1$ vector consists of Δy_{kjs}.The model is formulated as:

$$\boldsymbol{\Delta y_k} = \beta * \boldsymbol{C_k} + \boldsymbol{T_k} + \boldsymbol{e_k},$$

where $\beta * \boldsymbol{C_k}$, $\boldsymbol{T_k}$ and $\boldsymbol{e_k}$ are all defined in the same manner as those in the rQTc model above.

1.4.3 Double-Delta-QTc (ΔΔQTc)Model

The other model is the ΔΔQTc model, which is in good concordance with the primary endpoint "time-matched baseline adjusted QTc difference from placebo" defined in ICH E14. Garnett et al. (2008) presented the use of ΔΔQTc in concentration-QTc model and stated that it automatically accounted for the within-subject circadian rhythm effect, and Florin et al. (2011) explicitly described how to calculate the ΔΔQTc. This type of model is suitable for crossover SAD study since each subject has a placebo arm where ΔΔQTc can be computed. The procedure for calculating ΔΔQTc is as follows. First, QTc change from baseline (ΔQTc) is calculated, then the difference in ΔQTc between the drug period and corresponding placebo period is calculated and defined as the ΔΔQTc. We denote $\boldsymbol{j = p}$ as the placebo period and $\Delta\Delta\boldsymbol{y_{kjs}}$ as the ΔΔQTc value for subject $\boldsymbol{k}$ at time $\boldsymbol{t_s}$ in period $\boldsymbol{j}$ as follows:

$$\Delta\boldsymbol{y_{kjs}} = \boldsymbol{y_{kjs}} - \boldsymbol{y_{kj,-1}}, \qquad \boldsymbol{j \neq p},$$

$$\Delta\boldsymbol{y_{kps}} = \boldsymbol{y_{kps}} - \boldsymbol{y_{kp,-1}}, \qquad \boldsymbol{j = p},$$

$$\Delta\Delta\boldsymbol{y_{kjs}} = \Delta\boldsymbol{y_{kjs}} - \Delta\boldsymbol{y_{kps}}.$$

The ΔΔQTc is modeled as the following:

$$\boldsymbol{\Delta\Delta y_k = \beta * C_k + e_k.}$$

Here $\boldsymbol{\Delta\Delta y_k}$ is a $\boldsymbol{JT \times 1}$ vector consists of $\Delta\Delta\boldsymbol{y_{kjs}}$ for subject $\boldsymbol{k}$, while $\boldsymbol{\beta * C_k}$ and $\boldsymbol{e_k}$ denote the drug effect and variation associated with $\boldsymbol{\Delta\Delta y_k}$, respectively. Garnett et al. (2008) mentioned that the merit of the ΔΔQTc-type model is that it automatically accounted for the within-subject circadian rhythm. Thus in principal, we do not need to consider circadian rhythm effect in ΔΔQTc model here.

1.5 Dissertation Outline

This dissertation makes contributions to the concentration-QTc model from three aspects.

First, when researchers use the multi-oscillator functions to model the QTc circadian rhythm effect, usually several possible candidate functions are fitted simultaneously on the baseline/placebo data and the oscillator function with the best fit (quantified by smallest AIC or

BIC) is chosen to model the circadian rhythm effect. However in practice, drug concentration and ECG samples are measured in an unevenly spaced sampling strategy where the measurements are intensively measured around the Tmax (the time when concentration arrives the peak value) or waking hours of subjects, but are usually sparse elsewhere. Thus it is questionable whether the current sampling drug concentration/ECG sampling strategy can choose the right oscillator model or not. In Chapter 2, a comprehensive study is conducted to demonstrate the limitation of the current drug concentration/ECG sampling strategy and a possible modification is proposed, examined and validated via simulation studies.

Secondly, various types of concentration-QTc models have been proposed to evaluate the QTc prolongation by exploring the relationship between drug concentration and QTc prolongation, for which a brief introduction is given in Section 1.4. These concentration-QTc models are distinguished by their different ways in QTc baseline adjustment, which induces different efficiencies between models in estimating drug effect β – however such difference has not been studied yet. In Chapter 3, a comprehensive study is conducted to compare the efficiencies between these models, with the variance of the estimated drug effect for each model being derived to explain the differences in efficiencies between models. A novel conditional ΔQTc model (cΔQTc model) is proposed that is superior over other existing models in terms of having higher efficiency.

Lastly, methods other than the mixed effects models (MEM) analyzed in the Frequentist maximum likelihood (ML) approach are rarely mentioned in concentration-QTc modeling. Application of the Bayesian method is useful when we have reliable prior information on the parameters. When prior knowledge is not available, the Bayesian method with non-informative prior distributions is also viable and comparable to the Frequentist's approach. Comparison between the ML method and the Bayesian method will be evaluated via simulation studies.

Moreover, correctly specified covariance matrix structure is essential for the MEM with ML method but always hard to verify, the generalized estimating equations (GEE) and the generalized method of moments (GMM) do not impose distribution assumptions on the data and often produce consistent estimates when the covariance structure of data is mis-specified. Therefore, applications of the GEE and the GMM to concentration-QTc model are proposed and comparisons made to the MEM with the ML method.

Chapter 2 Evaluation of sampling strategies in identifying multi-oscillator functions

The QT/QTc interval data are known to be subject to within-day variation. As such, in the current practice of the concentration-QTc model, multi-oscillator functions are widely used to account for the circadian rhythm effect in QTc. The choice of oscillator functions among various candidate functions is typically based upon a comparison of some model fitting criteria, such as the AIC. A typical phase 1 single ascending dose (SAD) study collects only a limited number of ECG measurements throughout the course of a 24 hour period, with the large majority being measured during the waking hours or around the Tmax (the time when the maximal drug concentration happens), and very minimal measured during the nighttime hours or at time other than the Tmax. This conventional experimental design feature may affect the precision in identifying the true underlying oscillator function that may be generating the circadian rhythm effect in measurements. The potential impact of this important issue on evaluating QTc prolongation has drawn little attention in the literature. An evaluation of the potential limitations of the typical SAD design sampling strategy to identify the true underlying oscillator model is given in Chapter 2. Possible modifications in sampling strategy to improve the ability to identify the true underlying oscillator function are also proposed and examined via simulation.

2.1 Introduction to circadian rhythm effect of QT/QTc interval

Within-day variation (circadian rhythm) is a key characteristic of QT/QTc interval (Guo and Phyllis 2002). Evidence has shown that healthy subjects have an increase in QTc after food intake (Nagy et al. 1997), and QTc also tends to be higher at nighttime than in the daytime (Semetana et al. 2003, Kemal et al. 1999). In Smetana et al. (2003), ECG measurements were obtained on healthy volunteers every 30 seconds for a 24 hour interval. Their results clearly demonstrated a QTc circadian rhythm effect within a 24-hour interval. The QTc tends to be higher in nighttime than daytime for all the QT correction methods except for Bazett's method

(Figure 2 in Smetana et al. 2003). Molnar et al. (1996) also concluded that the QTc interval is longer during sleep, showing further that the QTc interval peaks shortly after awakening (Figure 4 in Molnar et al. 1996).

Since the phenomenon of the circadian rhythm effect is pronounced in monitoring the QTc intervals over the course of a 24-hour period, adjustment for circadian rhythm effect is typically done when evaluating the QTc prolongation with concentration-QTc model (Piotrovsky 2005, Rohatagi et al. 2009). The current method for modeling the QTc circadian rhythm effect and its potential limitations are introduced in Section 2.2.

2.2 Application of multi-oscillator function in modelling QTc circadian rhythm effect

2.2.1 Current method in modeling QTc circadian rhythm effect

In order to account for the known circadian rhythm of QT/QTc measurements over time, the multi-oscillator functions are routinely applied to the concentration-QTc model. These multi-oscillator functions typically include one or more cosine functions as previously described in Section 1.4.1. We revisit these functions here.

We denote y_{kjs} as the QTc value for subject k ($k = 1,2,\dots,K$) at time t_s ($s = 1,2,\dots,T$) of period j ($j = 1,2,\dots,J$). Let $\boldsymbol{y_k}$ be the $JT \times 1$ vector of the QTc value for subject k. Typically the $JT \times 1$ vector $\boldsymbol{T_k}$, consisting of the time effect T_{kjs} for subject k at time t_s in period j, is used to model the circadian rhythm effect (time effect), which is usually modeled by incorporation of a categorical time covariate in the model, or by using the multi-oscillator functions. The multi-oscillator function is formulated as follows.

$$T_{kjs} = QTc_0 * (1 + osc_{ks}),$$

$$osc_{ks} = amp24_k * \cos\left(\frac{2\pi*(t_s - acr24_k)}{24}\right) + amp12_k * \cos\left(\frac{2\pi*(t_s - acr12_k)}{12}\right) + amp6_k * \cos\left(\frac{2\pi*(t_s - acr6_k)}{6}\right). \quad (2.1)$$

The osc_{ks} part usually consists of 3 cosines functions, allowing for different effects over cosine periods of 24 hours, 12 hours and 6 hours. Each component has its own amplitude (amp24, amp12, and amp6, for 24, 12, and 6 hours, respectively) and phase (acr24, acr12, and

acr6 for 24, 12, and 6 hours, respectively). In some concentration-QTc models, all 3 cosine functions are included in the model, while some researchers only use one or two cosine functions in the model.

The model build-up and selection process when utilizing multi-oscillator functions to model the circadian rhythm effect in rQTc model and ΔQTc model consists of following steps. First, various multi-oscillator functions consisting of different combinations of cosine function components in (2.1) are fitted on the QTc (or ΔQTc) data from the placebo arm. Next, the multi-oscillator function that results in the best model fit (conventionally quantified by the model fit with the smallest AIC) is selected as the best model fit to the observed data. Finally, all the data (both placebo and treatment data) are pooled together to build the final model using the selected oscillator functions from the previous step. The flow chart of multi-oscillator function selection for fitting QTc circadian rhythm is shown in Figure 2.1.

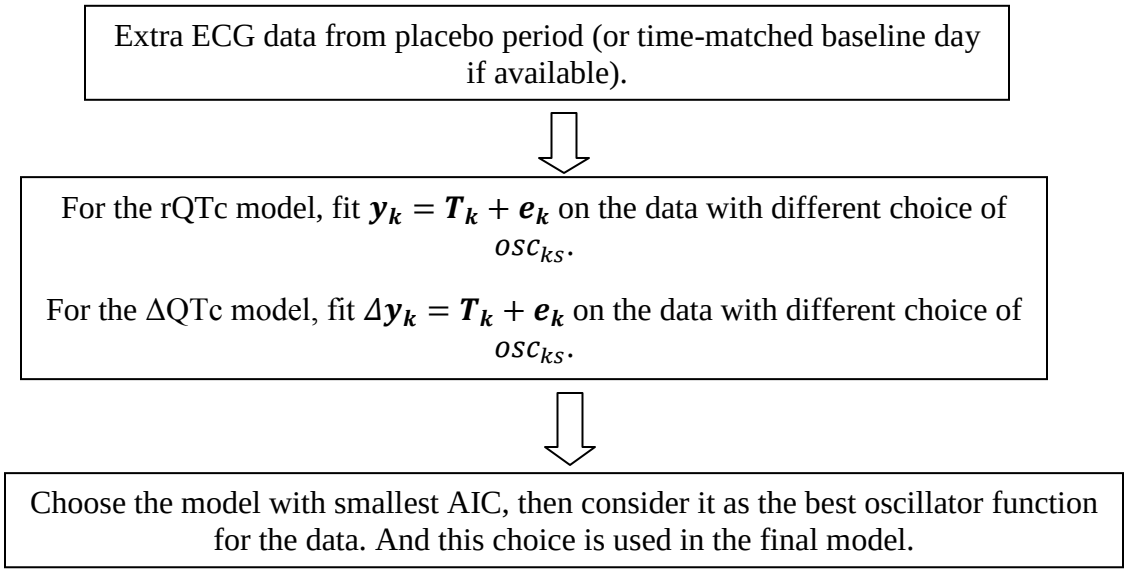

Figure 2.1 Typical procedures in choosing a multi-oscillator function in concentration-QTc model.

2.2.2 Current sampling strategy of SAD study

In order to better assess the drug-induced QTc interval prolongation, time points of ECG and drug concentration measurements in SAD studies are usually matched. Thus, the sampling

strategy of ECG measurements over a day is the same as the sampling strategy of drug concentrations.

In a typical SAD study, sampling time points of drug concentrations are generally designed to be distributed relatively intensively around the Tmax but can be relatively sparse at other time points. The number of sampling time points in waking time are also more than that during the nighttime considering the subjects' welfare. The ECG measurements are taken under the same sampling strategy as drug concentrations, and the sampling strategy are consistent over all periods. Therefore, the sampling strategy for ECG measurements is the same in placebo period as in the other periods.

Due to the unevenly spaced distributed sampling points, particularly between 12 and 24 hours, it is unclear whether the true underlying multi-oscillator function can be identified and estimated well. The possible consequences caused by the typical sampling strategy of SAD studies are discussed in the next section.

2.2.3 Potential issues of current sampling strategy in multi-oscillator function selection

The multi-oscillator function defined in formula (2.1) consists of 3 individual cosine functions, and we refer to them as 24hr-function, 12hr-function and 6hr-function based on cosine model differences. Each function has its own amplitude (amp24, amp12, amp6) which defines the maximum/minimum value of the cosine function, and its own phase (acr24, acr12, acr6) which defines the horizontal translation of cosine function comparing to a standard cosine function with phase of 0. The current sampling strategy may limit the ability to identify the true underlying multi-oscillator function, which is of course always unknown, from several aspects.

First, it may result in an inability to consistently distinguish various oscillator functions with different time length of cosine periods due to that the limited and unevenly spaced sampling time points in the 24-hour period. Since the time length between two adjacent measurements can be as large as 12 hours, which is problematic to distinguish between various oscillator functions. Second, the circadian rhythm effect we aim to estimate is only around 10ms, which is rather

small comparing to variation from other sources, such as random error, between-subject variation. Fitting a parametric model on the data may result in unstable parameter estimates of the true underlying oscillator functions.

2.3 Simulation study

One difficulty in evaluating the sampling strategy in identifying the true multi-oscillator function is that the true underlying model is not known, thus Monte Carlo simulation study would be an ideal method for this purpose.

The most widely used multi-oscillator function is a sum of 24hr-function and 12hr-function. Moreover, Smetana et al. (2003) and Molnar et al. (1996) both show that the QTc is relatively smooth in the daytime, and then reaching peak value at nighttime, thus the sum of the 24hour-function and the 12hour-function should be reasonable to reflect the QTc circadian rhythm effect (Figure 2.2).

Therefore, the sum of 24hr and 12hr models is used as the true model in the simulation to generate the QTc over time data and we investigate the chance that the true model is picked up by comparing the model fit criteria, AIC under the current sampling strategy.

Moreover, the modified sampling strategy will also be evaluated. As discussed in section 2.2, one of the potential issues of current sampling strategy is that the limited and unevenly distributed sampling points make some of the adjacent time points are as far as 12 hours away, thus it's not feasible to distinguish between the various multi-oscillator functions from this data. In reality, the sampling strategy is limited by many factors such as study cost, subject welfare, thus it may not be possible to add more time points, so we investigate whether we can modify the sampling strategy by rearranging the sampling points to make it more evenly distributed by moving one measurement in the 1st 12hour (2nd hour post-dose) to the 2nd 12hours (15th hour post-dose).

In simulation, the data are generated based on the true model,

$$y_{ks} = QTc_0 * \left(1 + amp24 * \cos\left(\frac{2\pi * (t_s - acr24)}{24}\right) + amp12 * \cos\left(\frac{2\pi * (t_s - acr12)}{12}\right)\right) + b_k + \varepsilon_{ks}$$

Parameters in the model are set as follows, amp24=-0.03, amp12=-0.016, acr24=6, acr12=0, $QTc_0 = 400$. The true underlying QTc along time used in the simulation is shown in the black line of Figure 2.2, the red line is the QTc along time under the current sampling strategy, and the blue line is the QTc along time under the modified sampling strategy.

Here b_k and ε_{ks} denote subject random effect and random error, respectively, and they are assumed to be independent with each other and both normally distributed with mean 0. We fix the total variance of between-subject variation and random error to be 300, and the variances of b_k and ε_{ks} are changed to investigate how they affect the identifiable rate. Typically, sample size for a FIH (first-in-man) "single ascending dose" study around 16, impact of larger sample size is also investigated. Simulation scenarios are summarized in Table 2.1. In each simulation, simulated data is generated based on the true model described above, and then all of the 6 candidate oscillator (Table 2.2) models are fitted using maximum likelihood (ML) method (Pinheiro and Bates 2006, Pinheiro et al. 2012). For each model, number of simulations achieve the smallest AIC among all the models is calculated, the portion of simulations with 24hr+12hr model achieving the smallest AIC is regarded as the identifiable rate of the true model under each simulation scenario. Simulation was run 1000 times under each simulation scenario.

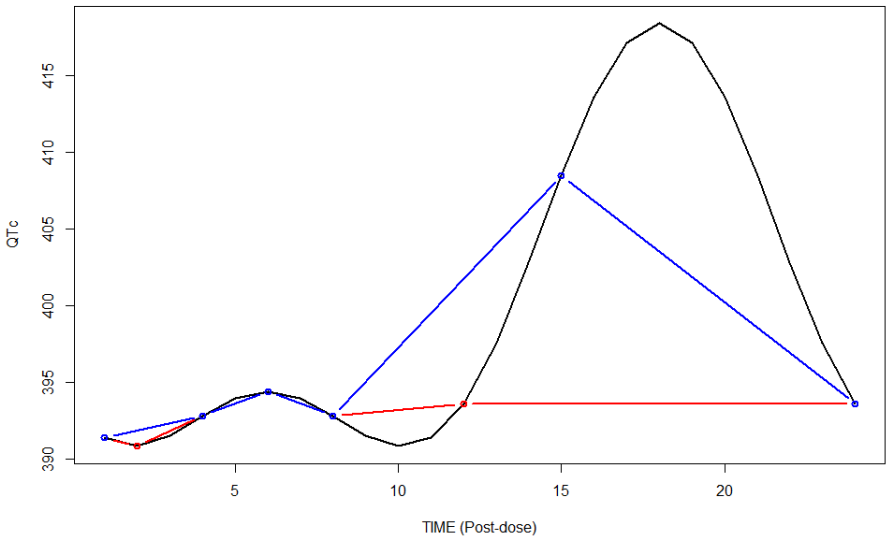

Figure 2.2 Mean QTc of true model with current sampling strategy (red) and modified sampling strategy (blue)

Table 2.1 Simulation scenarios in evaluating the current sampling strategy

Scenario	Sampling Strategy	Between-subject variance	Random error variance	Sample Size
1	1,2,4,6,8,12,24	240	60	16
2		180	120	
3		240	60	32
4		180	120	
5	1,4,6,8,12,15,24	240	60	16
6		180	120	
7		240	60	32
8		180	120	

Table 2.2 Summary of multi-oscillator functions included in the simulation

Model Type	Multi-oscillator function (osc)	Note
$y_{ks} = QTc_0 * (1 + osc) + b_k + \varepsilon_{ks}$, for subject k at time t_s.	$amp24 * \cos\left(\frac{2\pi * (t_s - acr24)}{24}\right) + amp12 * \cos\left(\frac{2\pi * (t_s - acr12)}{12}\right) + amp6 * \cos\left(\frac{2\pi * (t_s - acr6)}{6}\right)$	
	$amp12 * \cos\left(\frac{2\pi * (t_s - acr12)}{12}\right) + amp6 * \cos\left(\frac{2\pi * (t_s - acr6)}{6}\right)$	
	$amp24 * \cos\left(\frac{2\pi * (t_s - acr24)}{24}\right) + amp12 * \cos\left(\frac{2\pi * (t_s - acr12)}{12}\right)$	True model
	$amp24 * \cos\left(\frac{2\pi * (t_s - acr24)}{24}\right)$	
	$amp12 * \cos\left(\frac{2\pi * (t_s - acr12)}{12}\right)$	
	$amp6 * \cos\left(\frac{2\pi * (t_s - acr6)}{6}\right)$	

2.4 Simulation results

Simulation results are summarized in Table 2.3. The results clearly show that under the current sampling strategy (scenario 1-4), the identifiable rate of the true model is small. Increasing variance of random error reduces identifiable rate (1 vs 2, 3 vs 4), and increasing the sample size improves the recovery rate to some extent (1,2 vs 3,4), but the identifiable rate still

very low. For the modified sampling strategy, the identifiable rate is around 80%. Although increase in sample size and decrease in random error variance both improve the identifiable rate of the modified sampling strategy, but the impact of changes in sample size and random error variance is trivial comparing to the identifiable rate of the modified sampling strategy. Therefore, it can be concluded that the modified sampling strategy significantly improves the identifiable rate of the true model, and it is also robust to changes in sample size and random error variance in terms of consistent identifiable rate.

Table 2.3 Percentage of simulations with smallest AIC for each model

Scenario	24hr+12hr+6hr	12hr+6hr	24hr+12hr	24hr	12hr	6hr
1	3.60%	6.50%	***9.60%***	20.20%	22.80%	37.30%
2	2.20%	5.30%	***5.10%***	24.30%	23.90%	39.20%
3	3.40%	12.00%	***14.90%***	13.90%	19.50%	36.30%
4	2.80%	6.80%	***8.20%***	17.40%	22.50%	42.30%
5	13.70%	0.00%	***86.00%***	0.30%	0.00%	0.00%
6	13.30%	0.20%	***80.70%***	4.50%	0.20%	1.10%
7	14.20%	0.00%	***85.80%***	0.00%	0.00%	0.00%
8	13.90%	0.00%	***85.90%***	0.20%	0.00%	0.00%

The parameter estimates (Table 2.4) in each simulation scenario show that both sampling strategies provide unbiased estimates of oscillator function parameters, but the variance, and mean square error (MSE) both tend to be higher under the current sampling strategy when sample size and variance components are the same. This indicates that modified sampling strategy provides a more stable estimates of oscillator function parameters as we have expected.

In Figure 2.3, the current sampling strategy cannot reflect the peak area of QTc in the 24 hour interval but the modified sampling strategy can, thus it may raise a question that improvement in identifiable rate of modified sampling strategy is just a coincidence because it covers the peak area of QTc, not because modified sampling strategy make the unevenly spaced sampling strategy to be more evenly spaced.

To further confirm that improvement in identifiable rate is associated with evenly spaced sampling strategy, true model was modified by assigning 0.03 instead of -0.03 to amp24 so that the peak QTc area can be reflected by the current sampling strategy as well (Figure 2.3). But the identifiable rate is still low for the current sampling strategy (Table 2.5). This further confirms

that when the number of sampling points cannot be modified, to make the sampling time points more evenly distributed is essential in improving the identifiable rate of true oscillator model.

Table 2.4 Parameter estimates of true model on all the simulation scenarios

Scenario	QTc0			amp24		
	Mean	s.d.	MSE	Mean	s.d.	MSE
1	400.02	6.61	43.66	-0.03013	0.022564	0.000509
2	399.76	8.19	67.09	-0.02892	0.032113	0.001031
3	400.01	4.75	22.56	-0.02967	0.01624	0.000264
4	400.17	5.99	35.83	-0.03024	0.023354	0.000545
5	399.98	4.08	16.61	-0.03036	0.005742	3.31E-05
6	399.93	3.72	13.84	-0.03043	0.008256	6.83E-05
7	400.07	2.91	8.46	-0.03009	0.004414	1.95E-05
8	400.08	2.71	7.35	-0.03013	0.005945	3.53E-05
Scenario	amp12			acr24		
	Mean	s.d.	MSE	Mean	s.d.	MSE
1	-0.0165	0.01169	0.00014	6.071341	1.468837	2.160413
2	-0.0159	0.01684	0.00028	6.054165	1.806567	3.263355
3	-0.0161	0.00815	6.6E-05	5.988998	1.153124	1.328484
4	-0.0167	0.01205	0.00015	5.963482	1.205373	1.452805
5	-0.0167	0.0037	1.4E-05	5.940378	0.464877	0.219449
6	-0.0172	0.00511	2.8E-05	5.869026	0.740907	0.565548
7	-0.0163	0.00267	7.2E-06	5.959888	0.32079	0.104412
8	-0.0166	0.00375	1.4E-05	5.940691	0.468572	0.222858
Scenario	acr12					
	Mean	s.d.	MSE			
1	0.00145	0.85026	0.72222			
2	-0.0124	1.07768	1.16039			
3	0.06782	0.65176	0.42896			
4	0.03397	0.83944	0.70512			
5	-0.0497	0.52836	0.28135			
6	-0.1281	0.76282	0.59773			
7	-0.0409	0.3715	0.13954			
8	-0.0453	0.54748	0.30148			

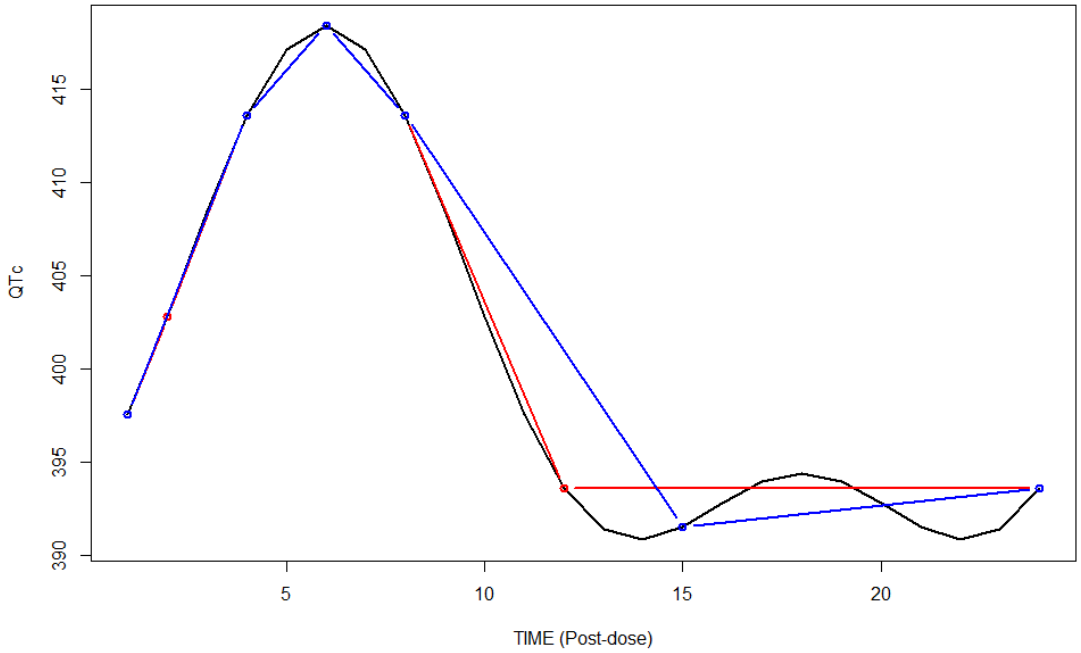

Figure 2.3 Mean QTc of true model with current sampling strategy (red) and modified sampling strategy (blue) with new true underlying oscillator function

Table 2.5 Percentage of simulations with smallest AIC for each model when peak QTc area is reflected in both sampling strategies

Scenario	24hr+12hr+6hr	12hr+6hr	24hr+12hr	24hr	12hr	6hr
1	3.10%	8.10%	***6.10%***	40.60%	42.10%	0.00%
2	2.40%	6.80%	***4.10%***	43.50%	43.20%	0.00%
3	4.20%	16.20%	***12.50%***	33.00%	34.10%	0.00%
4	3.20%	8.50%	***5.90%***	38.50%	43.90%	0.00%
5	13.70%	0.00%	***86.00%***	0.30%	0.00%	0.00%
6	13.50%	0.60%	***81.10%***	4.60%	0.20%	0.00%
7	14.20%	0.00%	***85.80%***	0.00%	0.00%	0.00%
8	13.90%	0.00%	***85.90%***	0.20%	0.00%	0.00%

2.5 Conclusion and discussion

To conclude, through comprehensive simulation study, the current sampling strategy of a typical SAD cannot guarantee that right oscillator function to be chosen to fit the QTc circadian rhythm effect. In order to improve the identifiable rate, the sampling strategy should be arranged as evenly spaced as possible, but it may not be feasible under some SAD study.

Moreover, another alternative would be totally ignoring the complicated multi-oscillator function, and use time as a categorical variable with linear mixed effects model instead, this method is more suitable for some SAD studies which fewer ECG measurements are available within a day so that oscillator functions would be evenly more problematic in modeling circadian rhythm effect. Hummer et al. (2015) mentioned to use time as a categorical variable to account for the QTc circadian rhythm effect and concluded that it will not reduce the precision in drug effect estimation evenly if the true underlying model is a multi-oscillator function. As the categorical time effect is more and more popular in concentration-QTc model, we will use the categorical time effect to model QTc circadian rhythm effect in the latter parts of this dissertation.

Chapter 3 Use of period-specific pre-dose baseline in concentration-QTc model for crossover SAD study

Various types of concentration-QTc models are used to explore the relationship between drug concentration and QTc prolongation. One of the essential differences in these concentration-QTc models with data from phase 1 crossover SAD study is how period-specific pre-dose QTc baseline are treated in the model. Different ways in treating the baseline QTc in concentration-QTc models result in different efficiencies in estimating the drug effect slope, and to our best knowledge, no studies have been conducted to compare the efficiencies of these models. In this chapter, rQTc model, ΔQTc model and ΔΔQTc model are briefly introduced, and the conditional ΔQTc (cΔQTc) model is proposed. Comprehensive study is conducted to compare efficiencies of the current models, the advantage of the proposed cΔQTc model over the existing models in terms of efficiency is also illustrated via derivations and simulation study.

3.1 Introduction to concentration-QTc models

Various concentration-QTc models are applied to quantify the relationship between drug concentration and QTc prolongation with data from phase 1 crossover single ascending dose (SAD) study. Typically, regression slope of QTc prolongation on drug concentration is the main parameter of interest. By estimating the drug effect slope and its variability, confidence interval of the slope is computed, together with the geometric mean of Cmax (maximal concentration) for a given dose, we are able to estimate the mean effect of QTc prolongation induced by a tested drug.

For concentration-QTc models with data from phase 1 crossover SAD study, different metrics in treating period-specific pre-dose QTc measurement and placebo period QTc measurements result in different types of concentration-QTc models. The most widely used models are rQTc model, ΔQTc model and ΔΔQTc model, an introduction to these models are presented in Section 1.4. All of the models share the same goal, which is to estimate the drug

effect slope, but they have different efficiencies in estimating the slope. The difference in efficiencies among different concentration-QTc models remain unclear. In this chapter, we aim to provide a comprehensive comparison on model efficiencies, and a novel conditional ΔQTc (cΔQTc) model is proposed which is more efficient than the other models. This chapter is formulated as follows. In Section 3.2, assumption and notation of the QTc data are introduced. Then the mean and variance-covariance structure of each concentration-QTc model is provided in Section 3.3. Section 3.4 and 3.5 present the derivations of the variance for the estimated drug effect slope for each model. We demonstrate our results via simulation studies in Section 3.6. Section 3.7 lends a general conclusion to Chapter 3.

3.2 General assumption and notation for concentration-QTc model comparison

Consider a phase 1 crossover single ascending dose (SAD) study with J periods, at each period, one pre-dose and T post-dose ECG measurements for subject k $(k = 1, \dots, K)$ are taken.

An example of a typical crossover SAD study with 4 periods and 16 subjects is summarized in Table 3.1. In this example, 8 doses of the tested drug are included in the study, with DOSE8 the highest while DOSE1 is the lowest. Each subject has 3 periods with different doses of drug and 1 period with placebo. At each period, drug concentrations are measured at selected time points within 24 hours after the first administration of drug/placebo, ECG measurements are usually taken at the same time points when drug concentrations are taken. Usually the number of post-dose time points is unevenly distributed as described in Chapter 2, with intensive distribution of time points around Tmax, and relatively sparsely distributed time points at other time.

For convenience of illustration, we will use $J = 4$ in Sections 3.2 and 3.3, and the conclusions reached using $J = 4$ can be easily extended to an SAD study with the number of periods other than 4.

In this study, we assume the QTc circadian rhythm effect is same for all the subjects, since phase 1 SAD study is an in-house study, factors affect the circadian rhythm effect are

controllable. We also assume that QTc prolongation is linearly dependent on drug concentration which is the most widely accepted relationship in the current concentration-QTc model. Lastly, we assume that QTc values is naturally different between subjects and can be quantified with a subject random effect, and QTc values are also different between periods within a subject which is quantified by a subject-by-period random effect and these two random effects are independent with each other.

Table 3.1 A typical design of a phase1 single ascending dose (SAD) study (DOSE1< DOSE2< DOSE3< DOSE4< DOSE5< DOSE6< DOSE7< DOSE8)

PANEL	**Number of Subjects**	**Period 1**	**Period 2**	**Period 3**	**Period 4**
A	N=2	Placebo	DOSE 3	DOSE 5	DOSE 7
	N=2	DOSE 1	Placebo	DOSE 5	DOSE 7
	N=2	DOSE 1	DOSE 3	Placebo	DOSE 7
	N=2	DOSE 1	DOSE 3	DOSE 5	Placebo
B	N=2	Placebo	DOSE 4	DOSE 6	DOSE 8
	N=2	DOSE 2	Placebo	DOSE 6	DOSE 8
	N=2	DOSE 2	DOSE 4	Placebo	DOSE 8
	N=2	DOSE 2	DOSE 4	DOSE 6	Placebo

Next, notations and assumptions of model comparisons are introduced.

Let scalar $y_{kj,-1}$ be the pre-dose QTc measurement for subject k in the jth period, y_{kjs} $(s = 1,2, \dots, T)$ is the post-dose QTc measurement k in the jth period at time t_s. Let $T \times 1$ vector $\boldsymbol{y_{kj}}$ be the T post-dose QTc measurements for subject k in the jth period $(j = 1, \dots, J)$ which consists of y_{kjs} $(s = 1,2, \dots, T)$.

Usually, we assume that the repeated-measures QTc data follow multivariate normal distribution for each subject and independent across subjects. The underlying model for subject k is

$$\begin{pmatrix} y_{k1,-1} \\ \boldsymbol{y_{k1}} \\ y_{k2,-1} \\ \boldsymbol{y_{k2}} \\ y_{k3,-1} \\ \boldsymbol{y_{k3}} \\ y_{k4,-1} \\ \boldsymbol{y_{k4}} \end{pmatrix} \sim N\left\{ \begin{pmatrix} \eta \\ \eta\mathbb{1} + \boldsymbol{\pi_{k1}} \\ \eta \\ \eta\mathbb{1} + \boldsymbol{\pi_{k2}} \\ \eta \\ \eta\mathbb{1} + \boldsymbol{\pi_{k3}} \\ \eta \\ \eta\mathbb{1} + \boldsymbol{\pi_{k4}} \end{pmatrix}, \boldsymbol{\Sigma} \right\}, \qquad (3.1)$$

where $\mathbb{1}$ is a vector of 1's, η is the grand mean QTc value at baseline measurement, and $\boldsymbol{\pi_{kj}} = (\pi_{kj1}, \pi_{kj2}, \dots, \pi_{kjT})'$, where $\pi_{kjs} = T_s + C_{kjs} * \beta$. Here T_s is the mean circadian rhythm effect on s-th post-dose QTc, C_{kjs} is the observed drug concentration for subject k in the jth period at time t_s. Therefore, $\beta * C_{kjs}$ is the drug induced QTc prolongation at drug concentration level of C_{kjs}. Also, β is the durg effect slope which quantifies the relationship between QTc prolongation and drug concentration.

Moreover, we do not consider period effect and other covariates, such as gender, age and BMI in the model here. We also assume that washout periods are sufficiently long so that no carryover effect is not considered. However, in the real concentration-QTc model, all the covariates that affect QTc should be considered and included in the model as appropriate. In model (3.1), $\boldsymbol{\Sigma}$ is the covariance matrix of the QTc measurements for subject k.

For model (3.1), the covariance matrix is formulated as follows. The covariance matrix is decomposed into within-period covariance matrix $\boldsymbol{\Sigma_w}$ and between-period covariance matrix $\boldsymbol{\Sigma_b}$ in the below form.

$$\boldsymbol{\Sigma} = \begin{pmatrix} \boldsymbol{\Sigma_w} & \boldsymbol{\Sigma_b} & \boldsymbol{\Sigma_b} & \boldsymbol{\Sigma_b} \\ \boldsymbol{\Sigma_b} & \boldsymbol{\Sigma_w} & \boldsymbol{\Sigma_b} & \boldsymbol{\Sigma_b} \\ \boldsymbol{\Sigma_b} & \boldsymbol{\Sigma_b} & \boldsymbol{\Sigma_w} & \boldsymbol{\Sigma_b} \\ \boldsymbol{\Sigma_b} & \boldsymbol{\Sigma_b} & \boldsymbol{\Sigma_b} & \boldsymbol{\Sigma_w} \end{pmatrix}_{4(T+1)\times 4(T+1)}$$

For within-period covariance matrix $\boldsymbol{\Sigma_w}$,

$$\boldsymbol{\Sigma_w} = cov\begin{pmatrix} y_{kj,-1} \\ \boldsymbol{y_{kj}} \end{pmatrix} = \begin{pmatrix} v_1 & \boldsymbol{\tau_1}' \\ \boldsymbol{\tau_1} & \boldsymbol{\Sigma_1} \end{pmatrix},$$

where v_1 is the variance of pre-dose QTc measurement, which can be different from the post-dose measurements if it is calculated as the mean value of several pre-dose QTc measurements,

however, here we assume there is only 1 pre-dose QTc measurement. In addition, $\boldsymbol{\tau_1}$ is the $T \times 1$ covariance vector between the pre-dose QTc measurement and post-dose QTc measurements, and $\boldsymbol{\Sigma_1}$ is the $T \times T$ covariance matrix for the post-dose QTc measurements in the same period.

For j and j' ($j \neq j'$), we have

$$\boldsymbol{\Sigma_b} = cov\left(\begin{pmatrix} y_{kj,-1} \\ \boldsymbol{y_{kj}} \end{pmatrix}, \begin{pmatrix} y_{kj',-1} \\ \boldsymbol{y_{kj'}} \end{pmatrix}\right) = \begin{pmatrix} v_2 & \boldsymbol{\tau_2}' \\ \boldsymbol{\tau_2} & \boldsymbol{\Sigma_2} \end{pmatrix},$$

v_2 is the covariance between pre-dose QTc measurements from different periods, $\boldsymbol{\tau_2}$ is the $T \times 1$ covariance vector between the pre-dose QTc measurement in a period and the post-dose QTc measurements in another period, $\boldsymbol{\Sigma_2}$ is the $T \times T$ covariance matrix between post-dose QTc measurements from different periods.

Let ${\sigma_s}^2$ denote the variance of subject random effect, ${\sigma_p}^2$ denote the variance of subject-by-period random effect, σ^2 denote the variance of random error, random effects and random error here are independent with each other. So that $v_1 = {\sigma_s}^2 + {\sigma_p}^2 + \sigma^2$, $\boldsymbol{\Sigma_1} = \left({\sigma_s}^2 + {\sigma_p}^2\right) * \boldsymbol{J_T} + \sigma^2 * \boldsymbol{I_T}$, $\boldsymbol{\tau_1} = ({\sigma_s}^2 + {\sigma_p}^2) * \mathbb{1}_{\boldsymbol{T}}$, $v_2 = {\sigma_s}^2$, $\boldsymbol{\Sigma_2} = {\sigma_s}^2 * \boldsymbol{J_T}$, $\boldsymbol{\tau_2} = {\sigma_s}^2 * \mathbb{1}_{\boldsymbol{T}}$, here $\mathbb{1}_{\boldsymbol{T}}$ is a $T \times 1$ matrix of 1's, J_T is a $T \times T$ matrix of 1's, I_T is the identity matrix. So the structure of $\boldsymbol{\Sigma}$ for each k is double compound symmetry.

Here, the covariance matrix structure we assume here is the same as Lu (2014) except that Lu (2014) used subject-by-time random effect to model the circadian rhythm effect while we use categorical time effect. By setting up the mean and covariance structure of the repeated measures QTc data, comparing efficiencies of different concentration-QTc models under the same data generation is meaningful.

Moreover, the correlation between two QTc measurements within the same day may reduce as the time points are further apart, which makes the double compound symmetry structure invalid in reality (Stylianou et al. 2008, Meng et al. 2010), thus it is important to access the robustness of models derived from the double compound symmetry structure in the presence of violation of covariance matrix structure. In the simulation part, scenarios with autoregressive with order 1 (AR(1)) random error structure is also evaluated, in these scenarios, $\boldsymbol{\Sigma_w} =$

$\left({\sigma_s}^2 + {\sigma_p}^2\right) * \boldsymbol{J}_{(\boldsymbol{T+1})} + \sigma^2 * \boldsymbol{\rho}_{(\boldsymbol{T+1})*(\boldsymbol{T+1})}$, where the (s, s')th element of $\boldsymbol{\rho}_{(\boldsymbol{T+1})*(\boldsymbol{T+1})}$ is $\rho^{|t_s - t_{s'}|}$, where t_s denotes time corresponding to s-th post-dose time point in each period.

In concentration-QTc model, the primary interest is to test if the upper bound of the drug-induced QTc prolongation is larger than 10 ms or not, and it is usually tested by the following hypothesis:

H_0: Upper bound of one-sides 95% confidence interval of drug induced QTc prolongation>10ms

VS

H_a: Upper bound of one-sides 95% confidence interval of drug induced QTc prolongation<10ms

(3.2)

Then the upper bound of one-sided 95% confidence interval of the drug-induced QTc prolongation is calculated as $C * U.B.of\ \beta$, where C is the Cmax (peak plasma concentration of a drug after administration) of a given dose and the dose should be available after SAD study when a therapeutic dose is determined, $U.B.of\ \beta$ is the upper bound of one-sided 95% confidence interval of estimated β calculated from the concentration-QTc model based on and the estimation of β and its standard error.

Based on the hypothesis, the important factor that affects the power of different concentration-QTc models is the variance of the estimate of β when all the models provide unbiased and consistent estimate of β, thus it is important to compare the variance of the estimated drug effect slope between different concentration-QTc models.

3.3 Mean and covariance matrix structure of each model

Section 3.2 presents the mean and covariance structure of the repeated measures QTc data from a SAD study, while in Section 3.3, the mean and covariance matrix structure of each concentration-QTc model are derived and presented.

3.3.1 Raw QTc (rQTc) model

Section 1.4.1 introduces the form of the rQTc model, which is as follows

$$\boldsymbol{y_k} = \beta * \boldsymbol{C_k} + \boldsymbol{T_k} + \boldsymbol{e_k}.$$

Under our model assumption, $\boldsymbol{y_k}$ is the $4T \times 1$ vector of the QTc value consists of y_{kjs}, $\boldsymbol{C_k}$ is the $4T \times 1$ vector and concentration value consists of C_{kjs} for subject k, respectively. Let $\boldsymbol{\Sigma_r}$ denote the covariance matrix for the rQTc model for each subject.

So for each subject k, we have

$$E(\boldsymbol{y_k}) = E\begin{pmatrix}\boldsymbol{y_{k1}}\\ \boldsymbol{y_{k2}}\\ \boldsymbol{y_{k3}}\\ \boldsymbol{y_{k4}}\end{pmatrix} = \begin{pmatrix}\eta\mathbb{1} + \boldsymbol{\pi_{k1}}\\ \eta\mathbb{1} + \boldsymbol{\pi_{k2}}\\ \eta\mathbb{1} + \boldsymbol{\pi_{k3}}\\ \eta\mathbb{1} + \boldsymbol{\pi_{k4}}\end{pmatrix},$$

$$\boldsymbol{\Sigma_r} = COV(\boldsymbol{y_k}) = COV\begin{pmatrix}\boldsymbol{y_{k1}}\\ \boldsymbol{y_{k2}}\\ \boldsymbol{y_{k3}}\\ \boldsymbol{y_{k4}}\end{pmatrix} = \begin{pmatrix}\boldsymbol{\Sigma_{wr}} & \boldsymbol{\Sigma_{br}} & \boldsymbol{\Sigma_{br}} & \boldsymbol{\Sigma_{br}}\\ \boldsymbol{\Sigma_{br}} & \boldsymbol{\Sigma_{wr}} & \boldsymbol{\Sigma_{br}} & \boldsymbol{\Sigma_{br}}\\ \boldsymbol{\Sigma_{br}} & \boldsymbol{\Sigma_{br}} & \boldsymbol{\Sigma_{wr}} & \boldsymbol{\Sigma_{br}}\\ \boldsymbol{\Sigma_{br}} & \boldsymbol{\Sigma_{br}} & \boldsymbol{\Sigma_{br}} & \boldsymbol{\Sigma_{wr}}\end{pmatrix}_{4T\times 4T},$$

The notations are the same as those in Section 3.2, where η is the grand mean QTc value, $\boldsymbol{\pi_{kj}} = (\pi_{kj1}, \pi_{kj2}, \dots, \pi_{kjT})'$, and $\pi_{kjs} = T_s + C_{kjs} * \beta$. In addition, $\boldsymbol{\Sigma_{wr}} = \boldsymbol{\Sigma_1}$ and $\boldsymbol{\Sigma_{br}} = \boldsymbol{\Sigma_2}$ as defined in Section 3.2.

In general, the covariance matrix follows double compound symmetry structure with ${\sigma_s}^2$ as the variance of subject random effect, ${\sigma_p}^2$ as the variance of subject-by-period random effect, σ^2 as the variance of random error.

In summary, under our model assumption, the rQTc model can be written as

$$y_{kjs} = \eta + \beta * C_{kjs} + T_s + b_k + b_{k(j)} + e_{kjs},$$

where b_k is the subject random effect, $b_{k(j)}$ is subject by period random effect, we usually assume that $b_k \sim N(0, {\sigma_s}^2)$, $b_{k(j)} \sim N(0, {\sigma_p}^2)$ and they are independent with each other. Therefore, the rQTc model includes time, drug concentration as fixed effects, and subject, subject-by-period as random effects.

3.3.2 ΔQTc model

Section 1.4.2 introduced the form of the ΔQTc model, which is as follows

$$\boldsymbol{\Delta y_k} = \beta * \boldsymbol{C_k} + \boldsymbol{T_k} + \boldsymbol{e_k}.$$

Here we denote QTc change from baseline as $\Delta \boldsymbol{y_{kjs}}$, and calculate which as

$$\Delta y_{kjs} = y_{kjs} - y_{kj,-1}.$$

Here $y_{kj,-1}$ is the baseline QTc measurement for subject k in period j, while $\boldsymbol{\Delta y_k}$ is a $4T \times 1$ vector consists of Δy_{kjs} for all j and s indices.

The change from pre-dose QTc vector for subject k is formulated as follows,

$$\boldsymbol{\Delta y_k} = \begin{pmatrix} \boldsymbol{\Delta y_{k1}} \\ \boldsymbol{\Delta y_{k2}} \\ \boldsymbol{\Delta y_{k3}} \\ \boldsymbol{\Delta y_{k4}} \end{pmatrix} = \begin{pmatrix} \boldsymbol{y_{k1}} - y_{k1,-1} * \mathbb{1}_{\boldsymbol{T}} \\ \boldsymbol{y_{k2}} - y_{k2,-1} * \mathbb{1}_{\boldsymbol{T}} \\ \boldsymbol{y_{k3}} - y_{k3,-1} * \mathbb{1}_{\boldsymbol{T}} \\ \boldsymbol{y_{k4}} - y_{k4,-1} * \mathbb{1}_{\boldsymbol{T}} \end{pmatrix}.$$

$$E(\boldsymbol{\Delta y_k}) = \begin{pmatrix} \boldsymbol{\pi_{k1}} \\ \boldsymbol{\pi_{k2}} \\ \boldsymbol{\pi_{k3}} \\ \boldsymbol{\pi_{k4}} \end{pmatrix},$$

where $\boldsymbol{\pi_{kj}}$ has the same definition as in (3.1), and

$$\boldsymbol{\pi_{kj}} = (\pi_{kj1}, \pi_{kj2}, \dots, \pi_{kjT})', \pi_{kjs} = T_s + C_{kjs} * \beta.$$

For each period j, we have

$$COV(\boldsymbol{\Delta y_{kj}}, \boldsymbol{\Delta y_{kj}})$$

$$= COV(\boldsymbol{y_{kj}}) + \mathbb{1}_{\boldsymbol{T}} * VAR(y_{kj,-1}) * \mathbb{1}_{\boldsymbol{T}}' - 2 * COV\left(\boldsymbol{y_{kj}}, (y_{kj,-1} * \mathbb{1}_{\boldsymbol{T}})\right)$$

$$= \boldsymbol{\Sigma_1} + v_1 * \boldsymbol{J_T} - 2 * ({\sigma_s}^2 + {\sigma_p}^2) * \boldsymbol{J_T} = \sigma^2 * (\boldsymbol{I_T} + \boldsymbol{J_T}).$$

For different periods j and j' ($j \neq j'$), we have

$$COV(\boldsymbol{\Delta y_{kj}}, \boldsymbol{\Delta y_{kj'}})$$

$$= COV\left(\left(\boldsymbol{y_{kj}} - y_{kj,-1} * \mathbb{1}_{\boldsymbol{T}}\right), \left(\boldsymbol{y_{kj'}} - y_{kj',-1} * \mathbb{1}_{\boldsymbol{T}}\right)\right)$$

$$= \boldsymbol{\Sigma_2} - \boldsymbol{\tau_2} * \mathbb{1}_{\boldsymbol{T}}{}' - \boldsymbol{\tau_2} * \mathbb{1}_{\boldsymbol{T}}{}' + \boldsymbol{\Sigma_2} = \mathbf{0}$$

Together we have

$$\boldsymbol{\Sigma_{\Delta}} = COV(\boldsymbol{\Delta y_k}) = COV\begin{pmatrix} \boldsymbol{\Delta y_{k1}} \\ \boldsymbol{\Delta y_{k2}} \\ \boldsymbol{\Delta y_{k3}} \\ \boldsymbol{\Delta y_{k4}} \end{pmatrix} = \begin{pmatrix} \boldsymbol{\Gamma} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{\Gamma} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \boldsymbol{\Gamma} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \boldsymbol{\Gamma} \end{pmatrix}_{4T \times 4T},$$

where $\boldsymbol{\Gamma} = \sigma^2 * (\boldsymbol{I_T} + \boldsymbol{J_T})$, $\boldsymbol{\Sigma_{\Delta}}$ is the covariance matrix of each subject for ΔQTc model.

In summary, under our model assumption, the ΔQTc model can be written as

$$\Delta y_{kjs} = \beta * C_{kjs} + T_s + b_{k(j)} + e_{kjs},$$

where $b_{k(j)}$ is subject by period random effect and $b_{k(j)} \sim N(0, \sigma^2)$ and they are independent with each other. Thus the ΔQTc model includes time, drug concentration as fixed effects, and subject-by-period as random effect.

3.3.3 ΔΔQTc model

Section 1.4.3 introduced the form of the ΔΔQTc model, which is as follows

$$\boldsymbol{\Delta\Delta y_k} = \boldsymbol{\beta} * \boldsymbol{C_k} + \boldsymbol{e_k}.$$

Here $\boldsymbol{\Delta\Delta y_k}$ is a $3T \times 1$ vector of $\Delta\Delta y_{kjs}$ for subject k. Furthermore, $\Delta\Delta y_{kjs}$ is calculated as follows ($j = p$ denotes the placebo period**)**:

$$\Delta \boldsymbol{y_{kjs}} = \boldsymbol{y_{kjs}} - \boldsymbol{y_{kj,-1}}, \qquad \boldsymbol{j \neq p},$$

$$\Delta \boldsymbol{y_{kps}} = \boldsymbol{y_{kps}} - \boldsymbol{y_{kp,-1}}, \qquad \boldsymbol{j = p},$$

$$\Delta\Delta \boldsymbol{y_{kjs}} = \Delta \boldsymbol{y_{kjs}} - \Delta \boldsymbol{y_{kps}}.$$

Without loses of generality, we assume period 1 is the placebo period, and thus the change from placebo adjusted for baseline vector is

$$\boldsymbol{\Delta\Delta y_k} = \begin{pmatrix} \boldsymbol{\Delta\Delta y_{k2}} \\ \boldsymbol{\Delta\Delta y_{k3}} \\ \boldsymbol{\Delta\Delta y_{k4}} \end{pmatrix} = \begin{pmatrix} \boldsymbol{\Delta y_{k2} - \Delta y_{k1}} \\ \boldsymbol{\Delta y_{k3} - \Delta y_{k1}} \\ \boldsymbol{\Delta y_{k4} - \Delta y_{k1}} \end{pmatrix} = \begin{bmatrix} \boldsymbol{-I_T} & \boldsymbol{I_T} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{-I_T} & \boldsymbol{0} & \boldsymbol{I_T} & \boldsymbol{0} \\ \boldsymbol{-I_T} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{I_T} \end{bmatrix} * \begin{pmatrix} \boldsymbol{\Delta y_{k1}} \\ \boldsymbol{\Delta y_{k2}} \\ \boldsymbol{\Delta y_{k3}} \\ \boldsymbol{\Delta y_{k4}} \end{pmatrix},$$

So $$\boldsymbol{E(\Delta\Delta y_k)} = E\begin{pmatrix} \boldsymbol{\Delta\Delta y_{k2}} \\ \boldsymbol{\Delta\Delta y_{k3}} \\ \boldsymbol{\Delta\Delta y_{k4}} \end{pmatrix} = \begin{pmatrix} \beta * \boldsymbol{C_{k2}} \\ \beta * \boldsymbol{C_{k3}} \\ \beta * \boldsymbol{C_{k4}} \end{pmatrix},$$

$$\boldsymbol{\Sigma_{\Delta\Delta}} = COV(\boldsymbol{\Delta\Delta y_k}) = COV\begin{pmatrix} \boldsymbol{\Delta\Delta y_{k2}} \\ \boldsymbol{\Delta\Delta y_{k3}} \\ \boldsymbol{\Delta\Delta y_{k4}} \end{pmatrix}$$

$$= \begin{bmatrix} \boldsymbol{-I_T} & \boldsymbol{I_T} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{-I_T} & \boldsymbol{0} & \boldsymbol{I_T} & \boldsymbol{0} \\ \boldsymbol{-I_T} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{I_T} \end{bmatrix} * \boldsymbol{\Sigma_\Delta} * \begin{bmatrix} \boldsymbol{-I_T} & \boldsymbol{I_T} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{-I_T} & \boldsymbol{0} & \boldsymbol{I_T} & \boldsymbol{0} \\ \boldsymbol{-I_T} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{I_T} \end{bmatrix}'$$

$$= \begin{bmatrix} \boldsymbol{2\Gamma} & \boldsymbol{\Gamma} & \boldsymbol{\Gamma} \\ \boldsymbol{\Gamma} & \boldsymbol{2\Gamma} & \boldsymbol{\Gamma} \\ \boldsymbol{\Gamma} & \boldsymbol{\Gamma} & \boldsymbol{2\Gamma} \end{bmatrix} = \sigma^2 * (\boldsymbol{I_{3T}} + \boldsymbol{J_{3T}} + \begin{bmatrix} \boldsymbol{J_T} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{J_T} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{J_T} \end{bmatrix} + \begin{bmatrix} \boldsymbol{I_T} & \boldsymbol{I_T} & \boldsymbol{I_T} \\ \boldsymbol{I_T} & \boldsymbol{I_T} & \boldsymbol{I_T} \\ \boldsymbol{I_T} & \boldsymbol{I_T} & \boldsymbol{I_T} \end{bmatrix}) \quad , \quad \text{where}$$

$\boldsymbol{\Gamma} = \sigma^2 * (\boldsymbol{I_T} + \boldsymbol{J_T})$.

From the above derivation, the ΔΔQTc model includes drug concentration as fixed effects, and subject, subject-by-period, subject-by-time as random effects. And the random effects and random error are independent with each other.

3.3.4 Conditional ΔQTc (cΔQTc) model

Lu (2014) proposed an analysis of covariance (ANCOVA) model for analyzing traditional crossover TQT study with period-specific pre-dose baseline, and his work incorporates the period-specific pre-dose QTc baseline and subject-averaged pre-dose QTc baseline in the model and demonstrates the efficiency of the proposed model. To our best knowledge, the use of baseline has not been addressed in the concentration-QTc models. In this section, we propose a conditional ΔQTc (cΔQTc) model which uses ΔQTc as the response

variable, and incorporate period-specific pre-dose QTc baseline and subject-averaged pre-dose QTc baseline as covariates in the model.

First, distribution of ΔQTc conditional on pre-dose baseline is derived. It is assumed that $((y_{k1,-1}, y_{k2,-1}, y_{k3,-1}, y_{k4,-1}), \boldsymbol{\Delta y_{k1}}', \boldsymbol{\Delta y_{k2}}', \boldsymbol{\Delta y_{k3}}', \boldsymbol{\Delta y_{k4}}')'$ follows a multivariate normal distribution and

$$COV\left(\left((y_{k1,-1}, y_{k2,-1}, y_{k3,-1}, y_{k4,-1}), \boldsymbol{\Delta y'_{k1}}, \boldsymbol{\Delta y'_{k2}}, \boldsymbol{\Delta y'_{k3}}, \boldsymbol{\Delta y'_{k4}}\right)'\right)$$

$$= \begin{pmatrix} \boldsymbol{\Sigma_{bb}} & \boldsymbol{\Sigma_{b\Delta}}' \\ \boldsymbol{\Sigma_{b\Delta}} & \boldsymbol{\Sigma_{\Delta}} \end{pmatrix}_{(4T+4)\times(4T+4)},$$

where $$\boldsymbol{\Sigma_{bb}} = COV\begin{pmatrix} y_{k1,-1} \\ y_{k2,-1} \\ y_{k3,-1} \\ y_{k3,-1} \end{pmatrix} = {\sigma_s}^2 * \boldsymbol{J_4} + \left({\sigma_p}^2 + \sigma^2\right) * \boldsymbol{I_4},$$

$$\boldsymbol{\Sigma_{\Delta}} = COV\begin{pmatrix} \boldsymbol{\Delta y_{k1}} \\ \boldsymbol{\Delta y_{k2}} \\ \boldsymbol{\Delta y_{k3}} \\ \boldsymbol{\Delta y_{k4}} \end{pmatrix} = \begin{pmatrix} \boldsymbol{\Gamma} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{\Gamma} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \boldsymbol{\Gamma} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \boldsymbol{\Gamma} \end{pmatrix}_{4T\times 4T}, \ \boldsymbol{\Gamma} = \sigma^2 * (\boldsymbol{I_T} + \boldsymbol{J_T}),$$

$$\boldsymbol{\Sigma_{b\Delta}} = COV\left(\begin{pmatrix} y_{k1,-1} \\ y_{k2,-1} \\ y_{k3,-1} \\ y_{k3,-1} \end{pmatrix}, \begin{pmatrix} \boldsymbol{\Delta y_{k1}} \\ \boldsymbol{\Delta y_{k2}} \\ \boldsymbol{\Delta y_{k3}} \\ \boldsymbol{\Delta y_{k4}} \end{pmatrix}\right)$$

$$= \begin{pmatrix} (-\boldsymbol{\sigma^2}) * \mathbb{1}_{\boldsymbol{T}} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & (-\boldsymbol{\sigma^2}) * \mathbb{1}_{\boldsymbol{T}} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & (-\boldsymbol{\sigma^2}) * \mathbb{1}_{\boldsymbol{T}} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & (-\boldsymbol{\sigma^2}) * \mathbb{1}_{\boldsymbol{T}} \end{pmatrix}_{4T\times 4}$$

$$= -\sigma^2 * \begin{pmatrix} \mathbb{1}_{\boldsymbol{T}} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbb{1}_{\boldsymbol{T}} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbb{1}_{\boldsymbol{T}} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbb{1}_{\boldsymbol{T}} \end{pmatrix}_{4T\times 4}.$$

Based on the property of multivariate normal distribution, the distribution of $\boldsymbol{\Delta y_k}$ condition on period-specific pre-dose QTc is also multivariate normal. Let $\boldsymbol{\Sigma_C}$ denote the

covariance matrix of $\boldsymbol{\Delta y_k}$ conditional on the pre-baseline QTc measurement for each subject. The covariance matrix is derived in the next step.

$$\boldsymbol{\Sigma_C} = COV\left(\begin{pmatrix}\boldsymbol{\Delta y_{k1}}\\ \boldsymbol{\Delta y_{k2}}\\ \boldsymbol{\Delta y_{k3}}\\ \boldsymbol{\Delta y_{k4}}\end{pmatrix}\middle|\begin{pmatrix}y_{k1,-1}\\ y_{k2,-1}\\ y_{k3,-1}\\ y_{k3,-1}\end{pmatrix}\right) = \boldsymbol{\Sigma_\Delta} - \boldsymbol{\Sigma_{b\Delta}\Sigma_{bb}}^{-1}\boldsymbol{\Sigma_{b\Delta}}'.$$

$\boldsymbol{\Sigma_{bb}}^{-1}$ can be calculated as follows (Dobin et al. 2005):

$$\boldsymbol{\Sigma_{bb}}^{-1} = (\sigma_s{}^2 * \boldsymbol{J_4} + (\sigma_p{}^2 + \sigma^2) * \boldsymbol{I_4})^{-1} = \frac{1}{\sigma_p{}^2+\sigma^2} * \boldsymbol{I_4} - \frac{\sigma_s{}^2}{(\sigma_p{}^2+\sigma^2)*(\sigma_p{}^2+\sigma^2+4\sigma_s{}^2)} * \boldsymbol{J_4}.$$

So that we have

$$\boldsymbol{\Sigma_{b\Delta}\Sigma_{bb}}^{-1}\boldsymbol{\Sigma_{b\Delta}}'$$

$$= \frac{\sigma^4}{\sigma_p{}^2+\sigma^2} * \begin{pmatrix}\boldsymbol{J_T} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0}\\ \boldsymbol{0} & \boldsymbol{J_T} & \boldsymbol{0} & \boldsymbol{0}\\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{J_T} & \boldsymbol{0}\\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{J_T}\end{pmatrix}_{4T\times 4\mathrm{T}} - \frac{\sigma_s{}^2 * \sigma^4}{(\sigma_p{}^2+\sigma^2) * (\sigma_p{}^2+\sigma^2+4\sigma_s{}^2)} * \boldsymbol{J_{4T*4T}}$$

$$\boldsymbol{\Sigma_C} = \boldsymbol{\Sigma_\Delta} - \boldsymbol{\Sigma_{b\Delta}\Sigma_{bb}}^{-1}\boldsymbol{\Sigma_{b\Delta}}'$$

$$= \sigma^2 * \boldsymbol{I_{4T*4T}} + \frac{\sigma^2*\sigma_p{}^2}{\sigma_p{}^2+\sigma^2} * \begin{pmatrix}\boldsymbol{J_T} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0}\\ \boldsymbol{0} & \boldsymbol{J_T} & \boldsymbol{0} & \boldsymbol{0}\\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{J_T} & \boldsymbol{0}\\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{J_T}\end{pmatrix}_{4T\times 4\mathrm{T}} + \frac{\sigma_s{}^2*\sigma^4}{(\sigma_p{}^2+\sigma^2)*(\sigma_p{}^2+\sigma^2+4\sigma_s{}^2)} * \boldsymbol{J_{4T*4T}}.$$

$$E\left(\begin{pmatrix}\boldsymbol{\Delta y_{k1}}\\ \boldsymbol{\Delta y_{k2}}\\ \boldsymbol{\Delta y_{k3}}\\ \boldsymbol{\Delta y_{k4}}\end{pmatrix}\middle|\begin{pmatrix}y_{k1,-1}\\ y_{k2,-1}\\ y_{k3,-1}\\ y_{k3,-1}\end{pmatrix}\right) = \begin{pmatrix}\boldsymbol{\pi_{k1}}\\ \boldsymbol{\pi_{k2}}\\ \boldsymbol{\pi_{k3}}\\ \boldsymbol{\pi_{k4}}\end{pmatrix} + \boldsymbol{\Sigma_{b\Delta}\Sigma_{bb}}^{-1} * \left(\begin{pmatrix}y_{k1,-1}\\ y_{k2,-1}\\ y_{k3,-1}\\ y_{k3,-1}\end{pmatrix} - E\begin{pmatrix}y_{k1,-1}\\ y_{k2,-1}\\ y_{k3,-1}\\ y_{k3,-1}\end{pmatrix}\right)$$

$$= \begin{pmatrix}\boldsymbol{\pi_{k1}}\\ \boldsymbol{\pi_{k2}}\\ \boldsymbol{\pi_{k3}}\\ \boldsymbol{\pi_{k4}}\end{pmatrix} + \boldsymbol{\Sigma_{b\Delta}\Sigma_{bb}}^{-1} * \left(\begin{pmatrix}y_{k1,-1}\\ y_{k2,-1}\\ y_{k3,-1}\\ y_{k3,-1}\end{pmatrix} - \begin{pmatrix}\eta\\ \eta\\ \eta\\ \eta\end{pmatrix}\right)$$

For subject k, the averaged pre-dose baseline across periods within subject can serve as an estimate for η. Kenward and Roger (2009) also mentioned that incorporating the averaged pre-dose baseline across periods within the same subject will reduce the between subject variation. Therefore the cΔQTc model includes drug concentration and time as fixed effects, and pre-dose QTc and the averaged pre-dose QTc across periods of the same subject as covariates. It has a double compound symmetry covariance structure with $\frac{\sigma^2 * {\sigma_p}^2}{{\sigma_p}^2+\sigma^2}$ as variance of subject-by-period random effect, $\frac{{\sigma_s}^2 * \sigma^4}{({\sigma_p}^2+\sigma^2)*({\sigma_p}^2+\sigma^2+4{\sigma_s}^2)}$ as the variance of subject random effect, σ^2 is the variance of the random error, and the random effects and random error are independent with each other.

3.4 $\boldsymbol{var(\hat{\beta})}$ of concentration-QTc models

Each of the concentration-QTc models in Section 3.3 belongs to the family of linear mixed effects model $\boldsymbol{Y = X\alpha + Z\gamma + \epsilon}$, where $\boldsymbol{Y}$ is the observed response vector, $\boldsymbol{X}$ is the design matrix of fixed effects, $\boldsymbol{Z}$ is the design matrix of random effects, $\boldsymbol{\alpha}$ is the unknown fixed effects while $\boldsymbol{\gamma}$ is the unknown random effects to be estimated, and $\boldsymbol{\epsilon}$ is the unobserved vector of random errors. Typically, the maximum likelihood (ML) method or restricted maximum likelihood (ML) method are used for parameter estimates in mixed effects model (Laird and Ware 1982), and the assumption for ML method is that $\boldsymbol{\gamma}$ and $\boldsymbol{\epsilon}$ both follow multivariate normal distribution with mean vector $\mathbf{0}$, and $\boldsymbol{var(\gamma) = G}$ while $\boldsymbol{var(\epsilon) = R}$. Let $\boldsymbol{V}$ denote the covariance matrix of $\boldsymbol{Y}$ then we have $\boldsymbol{V = ZGZ' + R}$.

The estimator $\boldsymbol{\hat{\alpha} = (X'\hat{V}^{-1}X)^{-}X'\hat{V}^{-1}Y}$ is the best linear unbiased estimator (BLUE) of $\boldsymbol{\alpha}$ if $\boldsymbol{\hat{V}}$ is known which is usually not true, thus we usually use the ML estimates $\boldsymbol{\hat{G}}$ and $\boldsymbol{\hat{R}}$ to calculate $\boldsymbol{\hat{V}}$**.** The approximate covariance matrix of $\boldsymbol{\hat{\alpha}}$ is $\boldsymbol{(X'\hat{V}^{-1}X)^{-}}$, therefore the variance of the estimated drug effect slope for each model can be derived by its design matrix of fixed effects, $\boldsymbol{X}$, and the estimated covariance matrix of the response vector, $\boldsymbol{\hat{V}}$. The ML estimator $\boldsymbol{\hat{V}}$ is a consistent estimator of $\boldsymbol{V}$, thus the variance of drug effect $\text{var}(\hat{\beta})$ for each model will be derived

based on $(\boldsymbol{X'V^{-1}X})^{-}$ instead of $(\boldsymbol{X'\widehat{V}^{-1}X})^{-}$ because we have prior knowledge of $\boldsymbol{V}$ under our model assumption.

In this section, the variance of the drug effect slope, $var(\hat{\beta})$, for each model is derived and compared. The power of each model to exclude a QTc prolongation effect in hypothesis testing (3.2) depends on $var(\hat{\beta})$, and comparisons of $var(\hat{\beta})$ between all models could provide a guideline on the efficiencies of each model. Subsequently it also indicates which model would have higher power to exclude a small QTc prolongation effect in hypothesis testing (3.2).

Before going any further, some notations are introduced first. Let $\boldsymbol{C} = \{\boldsymbol{C_{kjs}}\}$ be a $\boldsymbol{KJT}$-dimension vector whose element $\boldsymbol{C_{kjs}}$ denotes the observed drug concentration for subject $\boldsymbol{k}$ at period $\boldsymbol{j}$ and time $\boldsymbol{t_s}$. Let $\boldsymbol{C_{kj}}$ be the $\boldsymbol{T}$-dimension vector which denotes the observed drug concentration for subject $\boldsymbol{k}$ at period $\boldsymbol{j}$. We have:

$$\overline{\boldsymbol{C_{..}}} = \frac{\boldsymbol{1}}{\boldsymbol{KJ}}\sum_{\boldsymbol{k=1}}^{\boldsymbol{K}}\sum_{\boldsymbol{j=1}}^{\boldsymbol{J}}\boldsymbol{C_{kj}}, \qquad \overline{\boldsymbol{C_{k.}}} = \frac{\boldsymbol{1}}{\boldsymbol{J}}\sum_{\boldsymbol{j=1}}^{\boldsymbol{J}}\boldsymbol{C_{kj}}$$

$$\boldsymbol{C_{...}} = \sum_{\boldsymbol{k=1}}^{\boldsymbol{K}}\sum_{\boldsymbol{j=1}}^{\boldsymbol{J}}\sum_{\boldsymbol{t=1}}^{\boldsymbol{T}}\boldsymbol{C_{kjt}}, \quad \overline{\boldsymbol{C_{...}}} = \frac{\boldsymbol{1}}{\boldsymbol{KJT}}\boldsymbol{C_{...}},$$

$$\boldsymbol{C_{k..}} = \sum_{\boldsymbol{j=1}}^{\boldsymbol{J}}\sum_{\boldsymbol{t=1}}^{\boldsymbol{T}}\boldsymbol{C_{kjt}}, \quad \boldsymbol{C_{kj.}} = \sum_{\boldsymbol{t=1}}^{\boldsymbol{T}}\boldsymbol{C_{kjt}}$$

3.4.1 $var(\widehat{\boldsymbol{\beta_r}})$ of the rQTc model

From 3.3.1, let $\boldsymbol{X_r}$ denote the design matrix for the fixed effects with dimension $KJT \times (T+1)$, $\boldsymbol{V}_r$ denote the covariance matrix for the rQTc model with dimension $KJT \times KJT$, so

$$\boldsymbol{X_r} = \begin{bmatrix} \boldsymbol{I_T} & \boldsymbol{C_{11}} \\ \boldsymbol{I_T} & \boldsymbol{C_{12}} \\ \vdots & \vdots \\ \boldsymbol{I_T} & \boldsymbol{C_{kj}} \\ \vdots & \vdots \\ \boldsymbol{I_T} & \boldsymbol{C_{KJ}} \end{bmatrix} = [\boldsymbol{E} \quad \boldsymbol{C}], \text{ here } \boldsymbol{E} = \begin{bmatrix} \boldsymbol{I_T} \\ \boldsymbol{I_T} \\ \dots \\ \boldsymbol{I_T} \\ \dots \\ \boldsymbol{I_T} \end{bmatrix}, \boldsymbol{C} = \begin{bmatrix} \boldsymbol{C_{11}} \\ \boldsymbol{C_{12}} \\ \dots \\ \boldsymbol{C_{kj}} \\ \dots \\ \boldsymbol{C_{KJ}} \end{bmatrix},$$

$$\boldsymbol{V_r = I_K \otimes \Sigma_r, \Sigma_r = I_J \otimes \Omega + {\sigma_s}^2 * J_{JT\times JT}, \Omega = \sigma^2 * I_T + {\sigma_p}^2 * J_{T\times T}.}$$

Here $\boldsymbol{E}$ is a matrix of dimension $KJT \times T$ which is to model the time effect, and $\boldsymbol{C}$ is KJT-dimension vector which is the vector of the observed drug concentration.

The covariance matrix of the fixed effects can be rewritten as follows

$$(\boldsymbol{{X_r}'{V_r}^{-1}X_r})^{-1} = \begin{bmatrix} \boldsymbol{E'{V_r}^{-1}E} & \boldsymbol{E'{V_r}^{-1}C} \\ \boldsymbol{C'{V_r}^{-1}E} & \boldsymbol{C'{V_r}^{-1}C} \end{bmatrix}^{-1}$$

Our interested element, $var(\widehat{\beta_r})$, is the bottom right corner element of the matrix, where $\widehat{\beta_r}$ is the estimated drug effect slope by rQTc model. And according to Henderson et al. (1981), $var(\widehat{\beta_r})$ can be expressed as

$$\boldsymbol{(C'{V_r}^{-1}C - C'{V_r}^{-1}E * (E'{V_r}^{-1}E)^{-1} * E'{V_r}^{-1}C)^{-1},}$$

$$\boldsymbol{{V_r}^{-1} = I_K \otimes {\Sigma_r}^{-1}, {\Sigma_r}^{-1} = I_J \otimes \Omega^{-1} - \frac{{\sigma_s}^2}{(T*{\sigma_p}^2+\sigma^2)*(T*{\sigma_p}^2+\sigma^2+JT*{\sigma_s}^2)} J_{JT\times JT},}$$

(see Appendix 1.1)

$$\boldsymbol{(E'{V_r}^{-1}E)^{-1}} = \frac{1}{K}\left(\frac{1}{J}\boldsymbol{\Omega} + {\sigma_s}^2 \boldsymbol{J_{T\times T}}\right),$$

(see Appendix 1.2)

$$\text{so } \boldsymbol{{V_r}^{-1}E} = \mathbb{1}_{KJ} \otimes \boldsymbol{\Omega^{-1}} - \frac{{\sigma_s}^2 * J}{(T*{\sigma_p}^2+\sigma^2)*(T*{\sigma_p}^2+\sigma^2+JT*{\sigma_s}^2)} \boldsymbol{J_{KJT\times T}}$$

Further algebraic calculation can show that the variance for the estimated drug effect slope $var(\widehat{\beta_r})$ for the rQTc model (see Appendix 1.3) is formulated as,

$$\frac{1}{var(\widehat{\beta_r})} =$$

$$\sum_{k=1}^{K}\sum_{j=1}^{J}\left(\boldsymbol{C_{kj}} - \overline{\boldsymbol{C}_{..}}\right)'\boldsymbol{\Omega}^{-1}\left(\boldsymbol{C_{kj}} - \overline{\boldsymbol{C}_{..}}\right) - \frac{{\sigma_s}^2}{(T*{\sigma_p}^2+\sigma^2)*(T*{\sigma_p}^2+\sigma^2+JT*{\sigma_s}^2)}\sum_{k=1}^{K}(C_{k..} - \overline{C_{...}})^2$$

3.4.2 $var(\widehat{\boldsymbol{\beta}_{\Delta}})$ of the ΔQTc model

From Section 3.3.2, let $\boldsymbol{X_{\Delta}}$ denote the design matrix for the fixed effects for ΔQTc model with dimension $KJT \times (T+1)$, $\boldsymbol{V_{\Delta}}$ denote the covariance matrix for ΔQTc model with dimension $KJT \times KJT$, so that

$$\boldsymbol{X_{\Delta}} = \begin{bmatrix} \boldsymbol{I_T} & \boldsymbol{C_{11}} \\ \boldsymbol{I_T} & \boldsymbol{C_{12}} \\ \vdots & \vdots \\ \boldsymbol{I_T} & \boldsymbol{C_{kj}} \\ \vdots & \vdots \\ \boldsymbol{I_T} & \boldsymbol{C_{KJ}} \end{bmatrix} = [\boldsymbol{E} \quad \boldsymbol{C}], \boldsymbol{V_{\Delta}} = \boldsymbol{I_K} \otimes \boldsymbol{\Sigma_{\Delta}} = \boldsymbol{I_{KJ}} \otimes \boldsymbol{\Gamma}, \boldsymbol{\Gamma} = \sigma^2(\boldsymbol{I_T} + \boldsymbol{J_{T\times T}}).$$

Then we have

$$(\boldsymbol{X_{\Delta}}'\boldsymbol{V_{\Delta}}^{-1}\boldsymbol{X_{\Delta}})^{-1} = \begin{bmatrix} \mathbf{E}'\boldsymbol{V_{\Delta}}^{-1}\boldsymbol{E} & \boldsymbol{E}'\boldsymbol{V_{\Delta}}^{-1}\boldsymbol{C} \\ \boldsymbol{C}'\boldsymbol{V_{\Delta}}^{-1}\boldsymbol{E} & \boldsymbol{C}'V_{\Delta}^{-1}\boldsymbol{C} \end{bmatrix}^{-1}$$

Similarly to Section 3.4.1, $var(\widehat{\beta_{\Delta}})$ can be expressed as

$$(\boldsymbol{C}'\boldsymbol{V_{\Delta}}^{-1}\boldsymbol{C} - \boldsymbol{C}'\boldsymbol{V_{\Delta}}^{-1}\boldsymbol{E} * (\boldsymbol{E}'\boldsymbol{V_{\Delta}}^{-1}\boldsymbol{E})^{-1} * \boldsymbol{E}'\boldsymbol{V_{\Delta}}^{-1}\boldsymbol{C})^{-1}$$

$$\boldsymbol{V_{\Delta}}^{-1} = \boldsymbol{I_{KJ}} \otimes \boldsymbol{\Gamma^{-1}}, \ (\boldsymbol{E}'\boldsymbol{V_{\Delta}}^{-1}\boldsymbol{E})^{-1} = \frac{1}{KJ}\boldsymbol{\Gamma}, \boldsymbol{V_{\Delta}}^{-1}\boldsymbol{E} = \mathbb{1}_{KJ} \otimes \boldsymbol{\Gamma^{-1}}$$

Further algebraic calculation can show that the variance for the estimated drug effect slope $var(\widehat{\beta_{\Delta}})$ for the ΔQTc model is expressed as

$$\frac{1}{var(\widehat{\beta_{\Delta}})} = \sum_{k=1}^{K}\sum_{j=1}^{J}(\boldsymbol{C_{kj}} - \overline{\boldsymbol{C_{..}}})'\boldsymbol{\Gamma}^{-1}(\boldsymbol{C_{kj}} - \overline{\boldsymbol{C_{..}}}).$$

3.4.3 $var(\widehat{\boldsymbol{\beta}_{\Delta\Delta}})$ of the ΔΔQTc model

From Section 3.3.3, let $\boldsymbol{X_{\Delta\Delta}}$ denote the design matrix for the fixed effects for the ΔΔQTc model with dimension $K(J-1)T \times T$, $\boldsymbol{V_{\Delta\Delta}}$ denote the covariance matrix for the ΔΔQTc model with dimension $K(J-1)T \times K(J-1)T$, so that

$$\boldsymbol{X_{\Delta\Delta}} = \boldsymbol{C} = \begin{bmatrix} \boldsymbol{C_{11}} \\ \boldsymbol{C_{12}} \\ \cdots \\ \boldsymbol{C_{kj}} \\ \cdots \\ \boldsymbol{C_{KJ}} \end{bmatrix}$$

Usually, the data from placebo period are not included in the design matrix because the drug concentrations in placebo periods are supposed to be zero.

$$\boldsymbol{V_{\Delta\Delta}} = \boldsymbol{I_K} \otimes \boldsymbol{\Sigma_{\Delta\Delta}},\ \boldsymbol{V_{\Delta\Delta}}^{-1} = \boldsymbol{I_K} \otimes \boldsymbol{\Sigma_{\Delta\Delta}}^{-1}$$

$$\boldsymbol{\Sigma_{\Delta\Delta}} = (\boldsymbol{I_{J-1}} + J_{J-1}) \otimes \boldsymbol{\Gamma},\ \boldsymbol{\Sigma_{\Delta\Delta}}^{-1} = \boldsymbol{I_{J-1}} \otimes \boldsymbol{\Gamma^{-1}} - \frac{1}{J} * \boldsymbol{J_{J-1}} \otimes \boldsymbol{\Gamma^{-1}}$$

$$\boldsymbol{\Gamma} = \sigma^2(\boldsymbol{I_T} + \boldsymbol{J_{T\times T}}).$$

The covariance matrix of the fixed effects can be rewritten as follows

$$\left(\boldsymbol{X_{\Delta\Delta}}'\boldsymbol{V_{\Delta\Delta}}^{-1}\boldsymbol{X_{\Delta\Delta}}\right)^{-1} = 1/[\sum_{k=1}^{K}\sum_{j=1}^{J}\left(\boldsymbol{C_{kj}} - \overline{\boldsymbol{C_{k.}}}\right)'\boldsymbol{\Gamma}^{-1}\left(\boldsymbol{C_{kj}} - \overline{\boldsymbol{C_{k.}}}\right)]$$

So for the ΔΔQTc model, the variance for the estimated drug effect slope $var(\widehat{\beta_{\Delta\Delta}})$ satisfies the following equation:

$$\frac{1}{var(\widehat{\beta_{\Delta\Delta}})} = \sum_{k=1}^{K}\sum_{j=1}^{J}\left(\boldsymbol{C_{kj}} - \overline{\boldsymbol{C_{k.}}}\right)'\boldsymbol{\Gamma}^{-1}\left(\boldsymbol{C_{kj}} - \overline{\boldsymbol{C_{k.}}}\right).$$

3.4.4 $\boldsymbol{var(\widehat{\beta_c})}$ of the cΔQTc model

From Section 3.3.4, let $\boldsymbol{X_c}$ denote the design matrix for the fixed effects for cΔQTc model with dimension $KJT \times (T+3)$, $\boldsymbol{V_c}$ denote the covariance matrix for cΔQTc model with dimension $KJT \times KJT$, so that

$$\boldsymbol{X_c} = \begin{bmatrix} \boldsymbol{I_T} & \boldsymbol{P_{11}} & \boldsymbol{S_1} & \boldsymbol{C_{11}} \\ \boldsymbol{I_T} & \boldsymbol{P_{12}} & \boldsymbol{S_1} & \boldsymbol{C_{12}} \\ \cdots & \cdots & \cdots & \cdots \\ \boldsymbol{I_T} & \boldsymbol{P_{kj}} & \boldsymbol{S_k} & \boldsymbol{C_{ij}} \\ \cdots & \cdots & \cdots & \cdots \\ \boldsymbol{I_T} & \boldsymbol{P_{KJ}} & \boldsymbol{S_K} & \boldsymbol{C_{KJ}} \end{bmatrix}$$

Here, $\boldsymbol{P_{kj}} = y_{kj,-1} * \mathbb{1}_{\boldsymbol{T}}$ represent the period-specific pre-dose QTc baseline for period j of subject k. $\boldsymbol{S_k} = \frac{1}{J}\sum_{j=1}^{J} \boldsymbol{P_{kj}}$ is the average pre-dose QTc measurements across periods of subject k.

Let $\boldsymbol{V}_{\mathrm{c}} = \boldsymbol{I}_K \otimes \boldsymbol{\Sigma}_{\mathrm{c}}$, and from Section 3.3.4, $\boldsymbol{\Sigma}_{\mathrm{c}}$ is a double compound symmetry structure with $\frac{\sigma^2 * {\sigma_p}^2}{{\sigma_p}^2+\sigma^2}$ as the variance of the subject-by-period random effect, and $\frac{{\sigma_s}^2 * \sigma^4}{({\sigma_p}^2+\sigma^2)*({\sigma_p}^2+\sigma^2+J{\sigma_s}^2)}$ as the variance of the subject random effect, while σ^2 is the variance of the random error.

In Kenward and Roger (2009), the authors mentioned several ways in analyzing crossover trials that have within-period baseline measurements, and their analysis indicated that inclusion of fixed subject effect in the model will remove the between-subject information in the model. Inclusion of fixed subject effect in the model reduces the between-subject variance dramatically in our analysis. Therefore, for the sake of computational convenience, we assume that between-subject variance has negligible influence on the derivation of the variance of the estimated drug effect slope for the cΔQTc model $var(\widehat{\beta_c})$, so that $\widetilde{\boldsymbol{V}_{\mathrm{c}}}$ is used in the derivation instead of $\boldsymbol{V}_{\mathrm{c}}$ where

$$\widetilde{\boldsymbol{V}_{\mathrm{c}}} = \boldsymbol{I}_K \otimes \widetilde{\boldsymbol{\Sigma}_{\mathrm{c}}},\ \widetilde{\boldsymbol{\Sigma}_{\mathrm{c}}} = \boldsymbol{I}_J \otimes \left(\sigma^2 * \boldsymbol{I}_T + \frac{\sigma^2 * {\sigma_p}^2}{{\sigma_p}^2+\sigma^2} * \boldsymbol{J}_{T\times T}\right) = \boldsymbol{I}_J \otimes \boldsymbol{\gamma},$$

where we denote $\boldsymbol{\gamma} = \sigma^2 * \boldsymbol{I}_T + \frac{\sigma^2 * {\sigma_p}^2}{{\sigma_p}^2+\sigma^2} * \boldsymbol{J}_{T\times T}$.

Let $\boldsymbol{E_1} = \begin{bmatrix} \boldsymbol{I_T} & \boldsymbol{P_{11}} & \boldsymbol{S_1} \\ \boldsymbol{I_T} & \boldsymbol{P_{12}} & \boldsymbol{S_1} \\ \cdots & \cdots & \cdots \\ \boldsymbol{I_T} & \boldsymbol{P_{kj}} & \boldsymbol{S_k} \\ \cdots & \cdots & \cdots \\ \boldsymbol{I_T} & \boldsymbol{P_{KJ}} & \boldsymbol{S_K} \end{bmatrix} = [\boldsymbol{E} \quad \boldsymbol{B}]$, and $\boldsymbol{B} = [\boldsymbol{P} \quad \boldsymbol{S}]$, $\boldsymbol{P} = \begin{bmatrix} \boldsymbol{P_{11}} \\ \boldsymbol{P_{12}} \\ \cdots \\ \boldsymbol{P_{kj}} \\ \cdots \\ \boldsymbol{P_{KJ}} \end{bmatrix}$, $\boldsymbol{S} = \begin{bmatrix} \boldsymbol{S_1} \\ \boldsymbol{S_1} \\ \cdots \\ \boldsymbol{S_k} \\ \cdots \\ \boldsymbol{S_K} \end{bmatrix}$, thus the design matrix for cΔQTc model can be formulated as $\boldsymbol{X_c} = [\boldsymbol{E_1} \ \ \boldsymbol{C}]$.

Then we have $(\boldsymbol{X_c}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{X_c})^{-1} = \begin{bmatrix} \boldsymbol{E_1}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E_1} & \boldsymbol{E_1}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{C} \\ \boldsymbol{C}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E_1} & \boldsymbol{C}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{C} \end{bmatrix}^{-1}$, similarly, $var(\widehat{\beta_c})$ can be expressed as

$$(\boldsymbol{C}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{C} - \boldsymbol{C}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E_1} * (\boldsymbol{E_1}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E_1})^{-1} * \boldsymbol{E_1}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{C})^{-1}.$$

First, we will derive $(\boldsymbol{E_1}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E_1})^{-1}$,

$$(\boldsymbol{E_1}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E_1})^{-1} = \begin{bmatrix} \boldsymbol{E}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E} & \boldsymbol{E}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{B} \\ \boldsymbol{B}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E} & \boldsymbol{B}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{B} \end{bmatrix}^{-1} = \begin{bmatrix} \frac{1}{KJ}\boldsymbol{\gamma} + \overline{\boldsymbol{B}}_{\cdot\cdot} * \boldsymbol{INV} * \overline{\boldsymbol{B}}_{\cdot\cdot}' & -\overline{\boldsymbol{B}}_{\cdot\cdot} * \boldsymbol{INV} \\ -\boldsymbol{INV} * \overline{\boldsymbol{B}}_{\cdot\cdot}' & \boldsymbol{INV} \end{bmatrix},$$

where $\boldsymbol{INV} = \left(\sum_{k=1}^{K}\sum_{j=1}^{J}(\boldsymbol{B_{ij}} - \overline{\boldsymbol{B}}_{\cdot\cdot})'\boldsymbol{\gamma}^{-1}(\boldsymbol{B_{ij}} - \overline{\boldsymbol{B}}_{\cdot\cdot})\right)^{-1}$. (see Appendix 1.4)

Based on the results above, we have

$$\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E_1} * (\boldsymbol{E_1}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E_1})^{-1} * \boldsymbol{E_1}'\widetilde{\boldsymbol{V_c}}^{-1}$$

$$= \frac{1}{KJ}\boldsymbol{J_{KJ\times KJ}} \otimes \boldsymbol{\gamma}^{-1} + \widetilde{\boldsymbol{V_c}}^{-1}(\boldsymbol{B} - \boldsymbol{E} * \overline{\boldsymbol{B}}_{\cdot\cdot}) * \left[(\boldsymbol{B} - \boldsymbol{E} * \overline{\boldsymbol{B}}_{\cdot\cdot})'\widetilde{\boldsymbol{V_c}}^{-1}(\boldsymbol{B} - \boldsymbol{E} * \overline{\boldsymbol{B}}_{\cdot\cdot})\right]^{-1} * (\boldsymbol{B} - \boldsymbol{E} * \overline{\boldsymbol{B}}_{\cdot\cdot})'\widetilde{\boldsymbol{V_c}}^{-1}$$

$$= \frac{1}{KJ}\boldsymbol{J_{KJ\times KJ}} \otimes \boldsymbol{\gamma}^{-1} + \boldsymbol{SUM}$$

(see Appendix 1.5)

So we have

$$\boldsymbol{C}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{C} - \boldsymbol{C}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E_1} * (\boldsymbol{E_1}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E_1})^{-1} * \boldsymbol{E_1}'\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{C}$$

$$= \boldsymbol{C}'\left[\widetilde{\boldsymbol{V_c}}^{-1} - \frac{1}{KJ}\boldsymbol{J_{KJ\times KJ}} \otimes \boldsymbol{\gamma}^{-1}\right]\boldsymbol{C} - \boldsymbol{C}' * \boldsymbol{SUM} * \boldsymbol{C}$$

$$= \sum_{k=1}^{K}\sum_{j=1}^{J}\left(\boldsymbol{C}_{kj} - \overline{\boldsymbol{C}}_{\cdot\cdot}\right)'\boldsymbol{\gamma}^{-1}\left(\boldsymbol{C}_{kj} - \overline{\boldsymbol{C}}_{\cdot\cdot}\right) - \boldsymbol{C}' * \boldsymbol{SUM} * \boldsymbol{C}.$$

Based on practical experience, we found that $\boldsymbol{C}' * \boldsymbol{SUM} * \boldsymbol{C}$ has very little impact on derivation of $var(\widehat{\beta_c})$, a simulation in Section 3.4.5 will validate the impact of ignoring subject random effect $\frac{\sigma_s{}^2 * \sigma^4}{(\sigma_p{}^2+\sigma^2)*(\sigma_p{}^2+\sigma^2+\mathrm{J}*\sigma_s{}^2)}$ and $\boldsymbol{C}' * \boldsymbol{SUM} * \boldsymbol{C}$ in deriving $var(\widehat{\beta_c})$, therefore we have

$$\frac{1}{var(\widehat{\beta_c})} \approx \sum_{k=1}^{K}\sum_{j=1}^{J}(\boldsymbol{C}_{kj} - \overline{\boldsymbol{C}_{..}})' \boldsymbol{\gamma}^{-1} (\boldsymbol{C}_{kj} - \overline{\boldsymbol{C}_{..}}).$$

3.4.5 Validation of derived $var(\widehat{\beta_c})$ for the cΔQTc model

When deriving $var(\widehat{\beta_c})$ for the cΔQTc model in Section 3.4.4, an approximation is used by ignoring the between subject variation $\frac{\sigma_s{}^2 * \sigma^4}{(\sigma_p{}^2+\sigma^2)*(\sigma_p{}^2+\sigma^2+\mathrm{J}*\sigma_s{}^2)}$ of the cΔQTc model and $\boldsymbol{C}' * \boldsymbol{SUM} * \boldsymbol{C}$ in the derivation, finally we result in $var(\widehat{\beta_c}) \approx 1/\sum_{k=1}^{K}\sum_{j=1}^{J}(\boldsymbol{C}_{kj} - \overline{\boldsymbol{C}_{..}})' \boldsymbol{\gamma}^{-1} (\boldsymbol{C}_{kj} - \overline{\boldsymbol{C}_{..}})$. Simulation study below shows the approximation is reasonable.

We denote $var(\widehat{\beta_c})$ as the upper right element of $(\boldsymbol{X_c}' \boldsymbol{V}_{\mathrm{c}}{}^{-1} \boldsymbol{X_c})^{-}$ where we don't exclude the between subject variation and the $\boldsymbol{C}' * \boldsymbol{SUM} * \boldsymbol{C}$ term, while $\widetilde{var(\widehat{\beta_c})} = 1/\sum_{k=1}^{K}\sum_{j=1}^{J}(\boldsymbol{C}_{kj} - \overline{\boldsymbol{C}_{..}})' \boldsymbol{\gamma}^{-1} (\boldsymbol{C}_{kj} - \overline{\boldsymbol{C}_{..}})$. Details of drug concentration simulation process and QTc simulation is introduced in Section 3.6. In each simulation, percentage difference between the true asymptotic variance and the approximation $\frac{\widetilde{var(\widehat{\beta_c})} - var(\widehat{\beta_c})}{var(\widehat{\beta_c})} \times 100\%$ is calculated. From Table 3.2, in all the simulation scenarios, $\widetilde{var(\widehat{\beta_c})}$ tends to underestimate $var(\widehat{\beta_c})$, but the percentage difference is trivial (less than 4%), thus $1/\sum_{k=1}^{K}\sum_{j=1}^{J}(\boldsymbol{C}_{kj} - \overline{\boldsymbol{C}_{..}})' \boldsymbol{\gamma}^{-1} (\boldsymbol{C}_{kj} - \overline{\boldsymbol{C}_{..}})$ is a reasonable approximation of $var(\widehat{\beta_c})$.

Table 3.2 Summary of percentage difference between $var(\widehat{\beta_c})$ and $\widetilde{var(\widehat{\beta_c})}$ based on 1000 simulations

Scenario	${\sigma_s}^2$	${\sigma_p}^2$	σ^2	Mean	sd
1	240	15	45	-3.88%	1.29%
2	225	30	45	-2.59%	0.91%
3	210	45	45	-2.03%	0.80%
4	195	60	45	-1.71%	0.79%
5	180	30	90	-3.73%	1.16%
6	150	60	90	-2.51%	0.94%
7	120	90	90	-1.90%	0.84%
8	90	120	90	-1.52%	0.82%

3.5 Comparison of $\boldsymbol{var(\widehat{\beta})}$ between concentration-QTc models

In Section 3.4, variance of drug effect slope estimation for each model $\boldsymbol{var(\widehat{\beta_r})}$, $\boldsymbol{var(\widehat{\beta_\Delta})}$, $\boldsymbol{var(\widehat{\beta_{\Delta\Delta}})}$ and $\boldsymbol{var(\widehat{\beta_c})}$ are derived. As mentioned before, the variance of the estimated drug effect slope for each model directly relates to the power of each model to exclude a small QTc prolongation effect in hypothesis testing (3.2), therefore comparison of variance of estimated drug effect slope between all of the models will provide guidance on which model is the most efficient. In the current development of current concentration-QTc models, to our best knowledge, ΔQTc model is the most popular choice, thus we compare the other 3 variances to $\boldsymbol{var(\widehat{\beta_\Delta})}$.

3.5.1 $\boldsymbol{var(\widehat{\beta_r})}$ vs $\boldsymbol{var(\widehat{\beta_\Delta})}$

First we see that

$$\frac{1}{var(\widehat{\beta_r})} - \frac{1}{var(\widehat{\beta_\Delta})}$$

$= \frac{1}{T*{\sigma_p}^2+\sigma^2}\left[\frac{\sigma^2-{\sigma_p}^2}{\sigma^2(T+1)}\sum_{k=1}^{K}\sum_{j=1}^{J}\left(C_{kj.}-\frac{C_{...}}{KJ}\right)^2-\frac{{\sigma_s}^2}{\left(T*{\sigma_p}^2+\sigma^2+JT*{\sigma_s}^2\right)}\sum_{k=1}^{K}\left(C_{k..}-\frac{C_{...}}{K}\right)^2\right]$ (Appendix 1.6)

The rQTc model is more efficient than the ΔQTc model if $\frac{1}{var(\widehat{\beta_r})}-\frac{1}{var(\widehat{\beta_\Delta})}>0$, which means that

$$\frac{\left(T*{\sigma_p}^2+\sigma^2+JT*{\sigma_s}^2\right)}{\sigma^2(T+1)}\frac{\sigma^2-{\sigma_p}^2}{{\sigma_s}^2}>\frac{\sum_{k=1}^{K}\left(C_{k..}-\frac{C_{...}}{K}\right)^2}{\sum_{k=1}^{K}\sum_{j=1}^{J}\left(C_{kj.}-\frac{C_{...}}{KJ}\right)^2}$$

Since $\frac{\sum_{k=1}^{K}\left(Conc_{k..}-\frac{C_{...}}{K}\right)^2}{\sum_{k=1}^{K}\sum_{j=1}^{J}\left(Conc_{kj.}-\frac{C_{...}}{KJ}\right)^2}>0$, a necessary condition for this inequality holds true is ${\sigma^2}>{\sigma_p}^2$. Otherwise when ${\sigma^2}<{\sigma_p}^2$, $\frac{1}{var(\widehat{\beta_r})}-\frac{1}{var(\widehat{\beta_\Delta})}<0$ which indicates ${\sigma^2}<{\sigma_p}^2$ is a sufficient condition for ΔQTc model is more efficient than rQTc model.

3.5.2 $var(\widehat{\boldsymbol{\beta_{\Delta\Delta}}})$ vs $var(\widehat{\boldsymbol{\beta_{\Delta}}})$

First we have

$$\frac{1}{var(\widehat{\beta_\Delta})}-\frac{1}{var(\widehat{\beta_{\Delta\Delta}})}$$

$$=\sum_{k=1}^{K}\sum_{j=1}^{J}\left(\boldsymbol{C_{kj}}-\overline{\boldsymbol{C_{..}}}\right)'\boldsymbol{\Gamma^{-1}}\left(\boldsymbol{C_{kj}}-\overline{\boldsymbol{C_{..}}}\right)-\sum_{k=1}^{K}\sum_{j=1}^{J}\left(\boldsymbol{C_{kj}}-\overline{\boldsymbol{C_{k.}}}\right)'\boldsymbol{\Gamma^{-1}}\left(\boldsymbol{C_{kj}}-\overline{\boldsymbol{C_{k.}}}\right)$$

$$=\sum_{k=1}^{K}\sum_{j=1}^{J}\left(\overline{\boldsymbol{C_{k.}}}-\overline{\boldsymbol{C_{..}}}\right)'\boldsymbol{\Gamma^{-1}}\left(\overline{\boldsymbol{C_{k.}}}-\overline{\boldsymbol{C_{..}}}\right)>0$$

The last inequality holds true since $\boldsymbol{\Gamma^{-1}}$ is a positive definite matrix. This indicates that $var(\widehat{\beta_\Delta})$ is always less than $var(\widehat{\beta_{\Delta\Delta}})$, and the advantage is proportional to the between-subject drug concentration variation.

3.5.3 $var(\widehat{\beta_c})$ vs $var(\widehat{\beta_\Delta})$

Here we have

$$\frac{1}{var(\widehat{\beta_c})} - \frac{1}{var(\widehat{\beta_\Delta})}$$

$$\approx \sum_{k=1}^{K}\sum_{j=1}^{J}(C_{kj} - \overline{C_{..}})' \gamma^{-1} (C_{kj} - \overline{C_{..}}) - \sum_{k=1}^{K}\sum_{j=1}^{J}(C_{kj} - \overline{C_{..}})' \Gamma^{-1} (C_{kj} - \overline{C_{..}})$$

$$= \frac{1}{(T+1) * (T{\sigma_p}^2 + \sigma^2 + {\sigma_p}^2)} \sum_{k=1}^{K}\sum_{j=1}^{J}\left(C_{kj.} - \frac{C_{...}}{KJ}\right)^2 > 0$$

This indicates that $var(\widehat{\beta_c})$ is always less than $var(\widehat{\beta_\Delta})$. By including period-specific pre-dose baseline QTc and averaged pre-dose QTc across different periods within the same subject in cΔQTc model, between-subject variation and between-period within-subject variation are both reduced, and this also results in a decrease in variation when estimating the drug effect slope, thus $var(\widehat{\beta_c})$ is also reduced comparing to $var(\widehat{\beta_\Delta})$.

3.6 Simulation study

3.6.1 Simulation set-up

We use simulation studies to compare the efficiency of each model. The study design is summarized in Table 3.1, thus the number of subjects in the simulation is 16. 10mg, 50mg, 100mg, 150mg, 200mg, 300mg, 400mg, 600mg is assigned to dose1-dose8. Table 3.3 summarizes that models compared in the simulation study. Concentration models included in the comparison are summarized in Table 3.2.

To generate the repeated measures QTc data, model (3.1) in Section 3.2 is used with ${\sigma_s}^2$, ${\sigma_p}^2$ and σ^2 are the variance for subject random effect, subject-by-period random effect, and random error, respectively. Total variance is fixed at 300 and 8 scenarios are considered. In order

to investigate the robustness of each model when the within-day random error departure from CS structure, autoregressive with order 1 (AR(1)) within-day random error is simulated for each scenarios with 9 correlations, $\rho = 0.1, 0.2, \ldots, 0.8, 0.9$, as mentioned in Section 3.2, in these scenarios, in these scenarios, $\boldsymbol{\Sigma_W} = \left({\sigma_s}^2 + {\sigma_p}^2\right) * \boldsymbol{J_{(T+1)}} + \sigma^2 * \boldsymbol{\rho_{(T+1)*(T+1)}}$, where the (s, s')th element of $\boldsymbol{\rho_{(T+1)*(T+1)}}$ is $\rho^{|t_s - t_{s'}|}$, where t_s denotes time corresponding to the time point s in each period. The variance components for different simulation scenarios is described in Table 3.4.

In each period, 1 pre-dose QTc and 8 post-dose QTc measurements are available, the 8 post-dose QTc measurements are at 1st, 2nd, 4th, 6th, 8th, 12th, 15th, 24th hour post-dose. Mean QTc within each period is (397.6, 391.4, 390.8, 392.8, 394.4, 392.8, 393.6, 408.5, 393.6), which is calculated from the circadian rhythm model $400 * (1 - 0.03 * \cos\left(\frac{2\pi*(\mathrm{t}-6)}{24}\right) - 0.016 * \cos\left(\frac{2\pi*\mathrm{t}}{12}\right))$.

One-compartmental model is used to simulate drug concentration,

$$C_{kjs} = \frac{DOSE*ka_k}{V_k*(ka_k - ke_k)} * (\exp(-ke_k * t_s) - \exp(-ka_k * t_s)) * \exp(N(0, 0.1^2)),$$

$$ke_k = {CL_k}/{Vol_k}.$$

Here CL is the volume of plasma cleared of the drug per hour, Vol is the volume of distribution, ka is the infusion rate, we assume the pharmacokinetics parameter vary between different subjects, such that $CL_k = CL * \exp(b_1), V_k = V * \exp(b_2), ka_k = ka * \exp(b_3)$. Furthermore, b_1, b_2, b_3 are between-subject variability of all the population pharmacokinetics parameters and they are assumed to be distributed as $N(0, 0.2^2)$. CL is set to be 12 L/hr, Vol is set to be 60 L and ka is set to be 0.7 hr^{-1}.

The 600mg is chosen as the 'dose of interest'. The mean Cmax of 600mg based on the one-compartmental PK model is 6.05mg/L, thus for each of simulation scenario, we assign $\beta = 0.33$ to investigate the power of each model to rule out a small QTc prolongation effect, since when $\beta = 0.33$, the mean Cmax of 600mg induces a QTc prolongation of 2ms, which is less than regulatory concern of 10ms. Moreover, we assign $\beta = 1.65$ to investigate type-1 error

of each model, since the regulatory criteria to define a positive TQT study is that the upper bound of 1-sided 95% confidence interval of mean effect of drug induced QTc prolongation is larger than 10ms. When $\beta = 1.65$, the mean Cmax of 600mg induces a QTc prolongation of around 10ms, the type-1 error for each model at each scenario should be similar to make the power comparison meaningful. Each simulation is run 1000 times.

Table 3.3 Concentration-QTc models included in the simulation study

Model Type	Model Formulation	Covariance Matrix Structure
rQTc	$y_{kjs} = T_s + \beta * C_{kjs} + b_k + b_{k(j)} + \epsilon_{kjs}$	Double compound symmetry
ΔQTc	$\Delta y_{kjs} = T_t + \beta * C_{kjs} + b_{k(j)} + \epsilon_{kjs}$	Subject-by-period random effect
cΔQTc	$\Delta y_{kjs} = T_s + \beta * C_{kjs} + \beta_1 * y_{kj,-1} + \beta_2 * y_{k,-1} + b_k + b_{k(j)} + \epsilon_{kjs}$	Double compound symmetry
ΔΔQTc	$\Delta\Delta y_{kjs} = \beta * C_{kjs} + b_{k(j)} + b_{k(s)} + \epsilon_{kjs}$	Double compound symmetry with subject-by-time random effect

Table 3.4 Simulation scenarios and values of covariance parameters

Scenario	σ_s^2	σ_p^2	σ^2	ρ
1	240	15	45	0, 0.1, 0.2, …, 0.8, 0.9
2	225	30	45	
3	210	45	45	
4	195	60	45	
5	180	30	90	
6	150	60	90	
7	120	90	90	
8	90	120	90	

3.6.2 Hypothesis testing in the simulation study

As mentioned in Section 3.2, the hypothesis testing conducted for concentration-QTc model is

H_0: Upper bound of one-sides 95% confidence interval of drug induced QTc prolongation>10ms

VS

H_a: Upper bound of one-sides 95% confidence interval of drug induced QTc prolongation<10ms

For each model, upper bound of one-sided 95% confidence interval of the drug-induced QTc prolongation is calculated as $C * U.B.of\ \beta$, where $\boldsymbol{C}$ is the Cmax of a given dose and $U.B.of\ \beta$ is the upper bound one-sided 95% confidence interval of estimated drug effect slope for each model. Thus in each simulation, the geometric mean of Cmax at dose of 600mg is calculated as $\overline{Cmax}$, then U.B. of drug effect slope is calculated as $\hat{\beta} + qt(0.95, df) * sd(\hat{\beta})$, here the standard deviation of the estimated drug effect slope $sd(\hat{\beta})$ and degree of freedom are estimated based on restricted maximum likelihood estimate (REML) of each model (Bates et al. 2015).

In each simulation, percentage of simulations with $\overline{Cmax} * \left(\hat{\beta} + qt(0.95, df) * sd\left(\hat{\beta}\right)\right) < 10$ for each model is calculated. In the hypothesis testing above, it's considered as power to exclude a small QTc prolongation effect when the drug is "safe" and true $\beta = 0.33$, and it is considered as type-1 error when the drug is "not safe" and true $\beta = 1.65$.

3.6.3 Simulation results

Figure 3.1 and Figure 3.2 show the results of simulations from different scenarios.

In all the simulation scenarios, when the covariance structure is specified correctly for all the models (ρ=0), all the models have similar type-1 errors under each simulation scenario, no model has shown an advantage over the others when the drug is "not safe", the similar type-1 errors between models make the comparison of powers between models meaningful. The cΔQTc model always have the largest power to exclude a small QTc prolongation effect when the drug is "safe", while the power of ΔΔQTc model is always the lowest. These are consistent with our derivations that $var\left(\widehat{\beta_c}\right)$ is always smaller than $var\left(\widehat{\beta_\Delta}\right)$, and $var\left(\widehat{\beta_{\Delta\Delta}}\right)$ is always larger than $var\left(\widehat{\beta_\Delta}\right)$ in Section 3.5. The ΔQTc model is more powerful comparing to the rQTc model when

$\sigma_p{}^2$ is larger than σ^2(scenario 4 and 8), this is consistent with our conclusion in Section 3.5.1 that $\sigma_p{}^2 > \sigma^2$ is a sufficient condition for $var(\widehat{\beta_\Delta}) < var(\widehat{\beta_r})$, which indicates the ΔQTc model is more powerful than the rQTc model when $\sigma_p{}^2 > \sigma^2$. These results indicate that derivation and comparison of variance of estimated drug effect slope have been verified in the simulation study. Our proposed cΔQTc model always performs better than the other models in terms of higheer power to exclude a small drug prolongation effect when the drug is "safe".

When the AR(1) structure is imposed on the data, the cΔQTc model still has the largest power in almost all the scenarios. Type-1 error of all models increase comparing to the simulation scenarios when the covariance structure is correctly specified (ρ=0), but no model has shown advantage over the others in terms of a smaller type-1 error when the random errors within the same period for a subject is AR(1) structure. The rQTc model seems to be affected most by AR(1) within-period random error structure as it has the largest type-1 error in all the scenarios when the within-subject random error correlation is large (ρ>0.7).

3.7 Conclusion and discussion

Different concentration-QTc models have been applied by researchers without explaining the rationale behind them. For concentration-QTc model with data from phase 1 crossover SAD study, the differences among models are mainly based on how to treat the period-specific pre-dose QTc baseline in each model. In chapter 3, we compare the efficiencies of each model by assuming the covariance matrix structure of the QTc measurements is double compound symmetry, and the period-specific pre-dose QTc baseline has the same distribution as the post-dose QTc. The cΔQTc model is proposed which reduces the variance of the estimated drug effect slope by including period-specific pre-dose QTc baseline and subject-averaged pre-dose QTc baseline as covariates in the model. We conclude our proposed cΔQTc model is most efficient in detecting a safe drug comparing to the existing models via derivations of the variance of drug effect slope and intensive simulation.

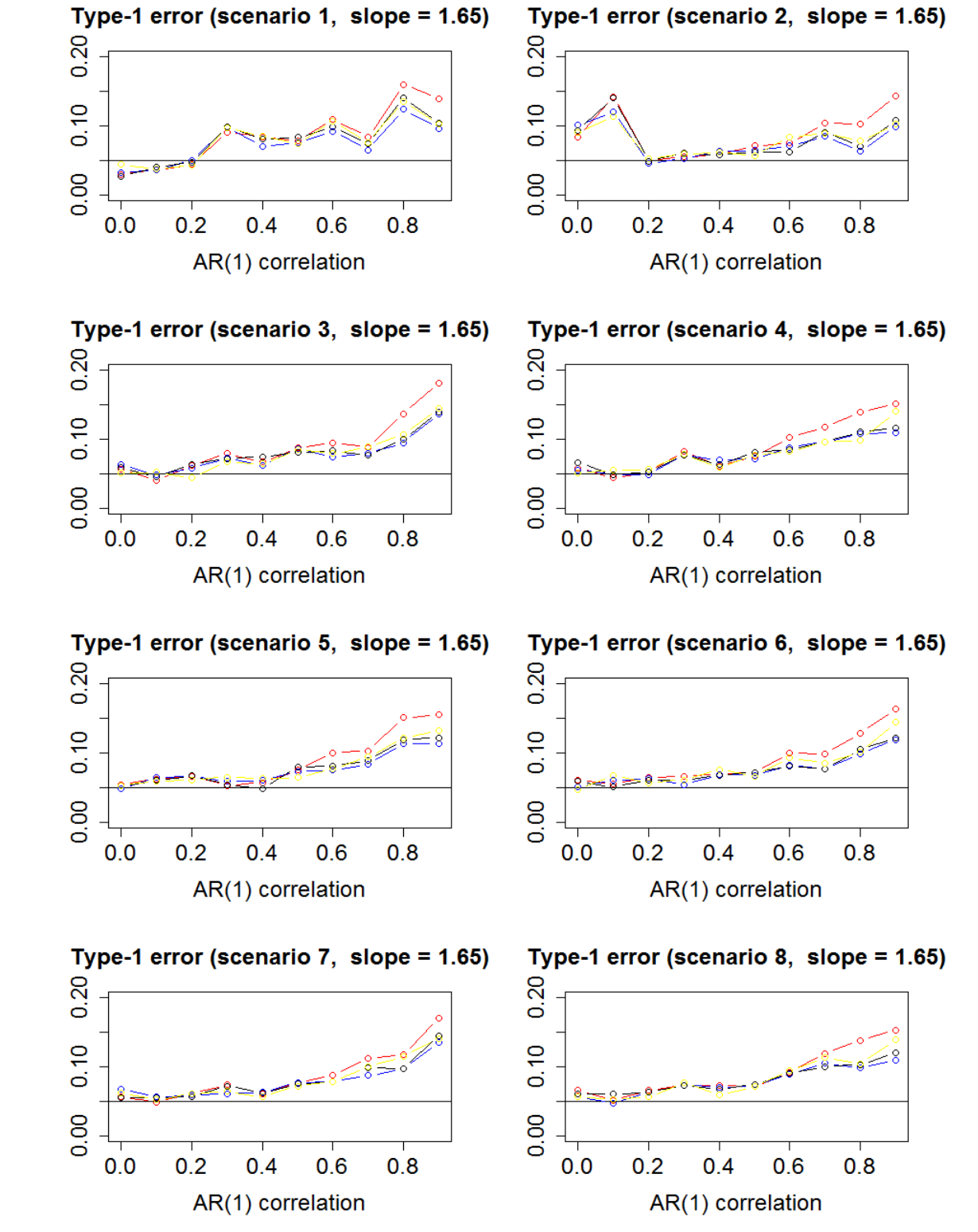

Figure 3.1 Simulation results (type-1 error) in model comparison

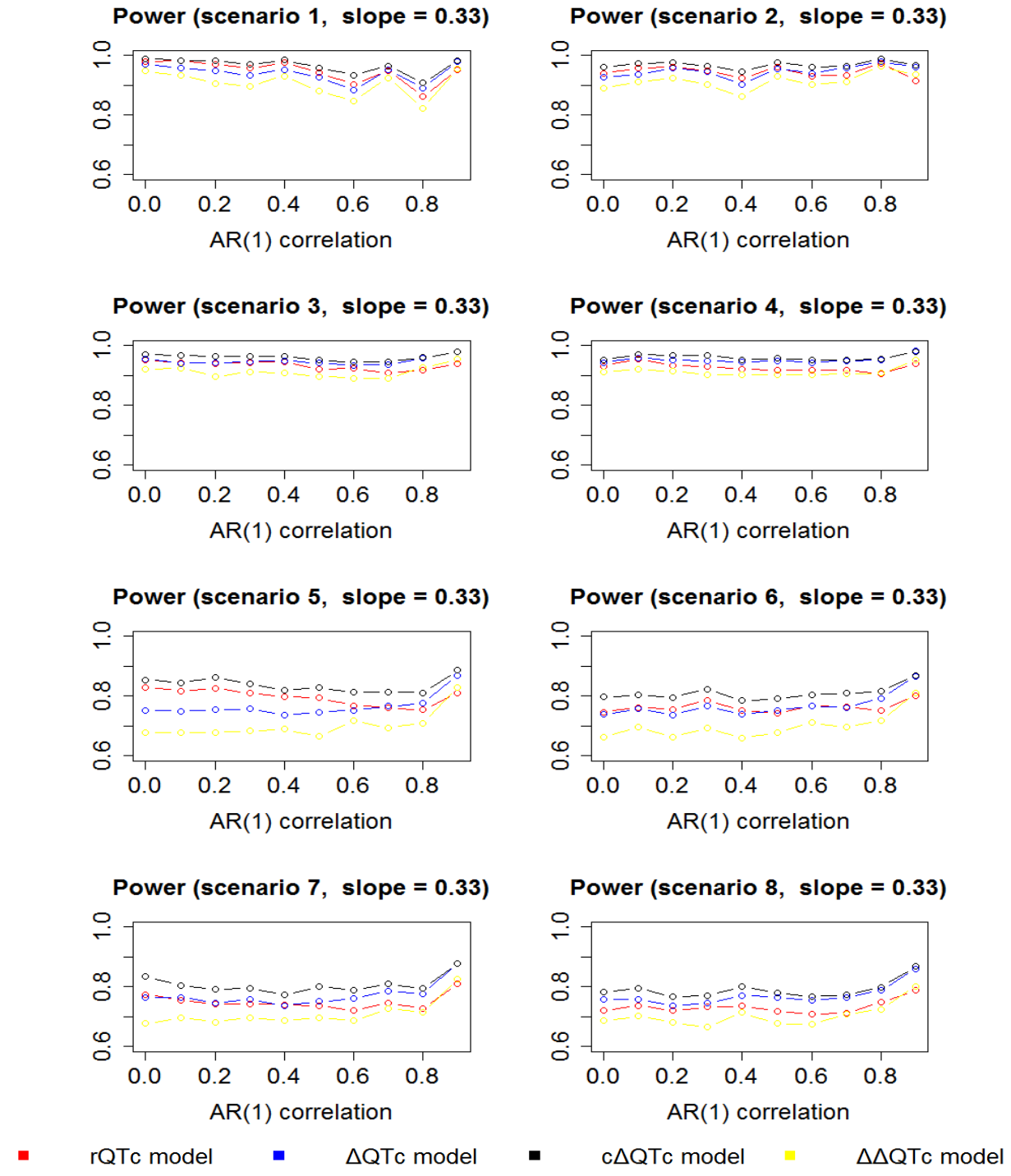

Figure 3.2 Simulation results (power) in model comparison

Chapter 4 Application of Bayesian method, GEE and GMM in concentration-QTc model

The mixed effects model analyzed with the maximum likelihood (ML) method is widely used in parameter estimation and statistical inference of the concentration-QTc model. In contrast, other methods, such as the mixed effects model in a Bayesian framework, the Generalized Estimating Equations (GEE) and the Generalized Methods of Moments (GMM), are rarely addressed. In this chapter, applications of the mixed effects model with the Bayesian method, the GEE and the GMM in the concentration-QTc model are proposed and discussed. For the purpose of illustration of the novel applications of these methods, the rQTc model and its counterparts of the above methods are used in the entire chapter.

4.1 Mixed effects model with the Bayesian method

4.1.1 Introduction to mixed effects model with the Bayesian method

With the rapid development of computation resource, when estimating parameters of the mixed effects model, some advanced but computationally intensive method other than the maximum likelihood (ML) method, such as the Bayesian method, begins to gain popularity. The Bayesian method automatically takes prior knowledge of parameters into consideration, it applies the Bayes' theorem on parameters' prior distributions and the likelihood function to produce posterior distribution of the parameters. Inferences on the parameters are performed on the derived posterior distribution. Gibbs sampler, which belongs to Markov chain Monte Carlo (MCMC) algorithm, is among the most popular methods to approximate a desired multivariate probability distribution when the direct sampling is difficult (Casella and Edward 1992).

In the research of concentration-QTc model, linear mixed effects model with the maximum likelihood (ML) method is widely used, but the Bayesian method is rarely mentioned.

Chain et al. (2011) has provided a framework of the Bayesian mixed effects model parameter estimate and inference of concentration-QTc model, they used non-informative prior distributions for all the parameter of interests and then inferences on parameters were made on the derived posterior distribution. In their work, comparison of the Bayesian method and the ML method has not been addressed. Browne et al. (2006) compared mixed effects model with the ML method and the Bayesian method with non-informative prior distributions via simulation studies. They concluded that in a mixed effect model with only subject random effect on intercept, the Bayesian method is better than the ML method in terms of higher coverage of the small random effect variance by confidence interval, especially when the intra-class correlation is small, although they both cannot achieve the nominal coverage. When prior distribution is available on parameters of interest, the Bayesian method can use this information in parameter estimate and inference while the ML method cannot, but how different they are when reliable prior information on parameters are available are not clear. Finally we do add the cautionary note that with a wrongly specified prior, the Bayesian method can produce misleading results.

In Section 4.1, an application of the Bayesian method on the concentration-QTc model with normality assumptions on random effects and random error is proposed. Moreover, a comparison between the Bayesian method and the ML method is discussed.

4.1.2 Application of the Bayesian method in concentration-QTc model

As described in Chapter 3, the rQTc model with data from crossover single ascending dose (SAD) study is formulated as follows,

$$y_{kjs} = T_s + \beta * C_{kjs} + b_k + b_{k(j)} + \epsilon_{kjs}.$$

Here y_{kjs} denotes the QTc value for subject k at time t_s of period j, T_s is the categorical time effect at time t_s, C_{kjs} is the observed drug concentration for subject k at time t_s of period j, b_k is the subject random effect and $b_{k(j)}$ is subject-by-period random effect, and ϵ_{kjt} is the i.i.d. random error. In mixed effects model with the ML method and the Bayesian method, we always assume that the random effects and random error are normally distributed, such that,

$b_k \sim N(0, {\sigma_s}^2)$, $b_{k(j)} \sim N(0, {\sigma_p}^2)$, $\epsilon_{kjt} \sim N(0, \sigma^2)$ and the random effects and random error here are all independent with each other.

Now we introduce how to estimate the parameters with the Bayesian method. Given $y_{kjs}|\beta, T_s, {\sigma_s}^2, {\sigma_p}^2, \sigma^2 \sim N\left(T_s + \beta * C_{kjs} + b_k + b_{k(j)}, \sigma^2\right)$, the non-informative priors are assigned to the parameters as follows,

$$\beta \sim N(0{,}1000000), \quad T_s \sim N(0{,}1000000) \text{ for each } s,$$

$$1/{\sigma_s}^2 \sim Gamma(0.001{,}0.001),\ 1/{\sigma_p}^2 \sim Gamma(0.001{,}0.001),\ 1/\sigma^2 \sim Gamma(0.001{,}0.001)$$

The shapes of the non-informative prior distribution for the parameters are almost flat, thus it has no preferences on specific values for a specific parameter, which is similar to the ML method. Based on the prior distributions, posterior distribution of each parameter is generated via Gibbs sampler using JAGS (Plummer 2003). Inference on each parameter is then performed using the derived posterior distribution. When we have reliable information on some of the parameters, the non-informative prior distributions described above are replaced with the available prior distributions of those parameters.

4.1.3 Comparison between the Bayesian method with non-informative prior distributions and the ML method in concentration-QTc model

Browne and Draper (2006) compared the ML method and the Bayesian method with non-informative prior distributions in a 2-level hierarchical model with only subject random effect on the intercept. Their simulation study indicated that these two methods are very similar in terms of bias of fixed effects estimation, but the confidence interval coverage of the small random effect variance is higher in the Bayesian method especially when the intra-class correlation is small. The rQTc model with data from crossover SAD study can be consider a 3-level hierarchical model, thus we will investigate if the better coverage of small random effect variance for the Bayesian method with non-informative prior distributions still holds, and further comparison between the ML method and the Bayesian method with non-informative prior distributions in

terms of power and type-1 error in the hypothesis testing conducted in the concentration-QTc model will also be investigated.

We use simulation studies to compare the two methods. The study design and QTc data simulation procedures are the same as those in Chapter 3. Simulation scenario is summarized in Table 4.1. Here in order to compare the Bayesian method with non-informative prior distributions and the ML method on their confidence interval coverage of the random effects variances, we intentionally set the variance of subject random effect and the variance of subject-by-period random effect to be small.

In each simulation, the 95% confidence interval for each covariance parameter are computed, coverage of the true parameter for each method is computed as the number of simulations that the 95% confidence interval of the parameter estimate covers the true value of the parameter, and also the estimated random effect variance is not 0. Similarly as in chapter 3, the type-1 error and power in each scenario are also computed and compared between the two methods. The number of simulation for each scenario is 1000.

Table 4.1 Simulation scenarios for comparison between the Bayesian method with non-informative prior distributions and the ML method

Scenario	σ_s^2	σ_p^2	σ^2
1	5	205	90
2	10	200	90
3	15	195	90
4	20	190	90
5	25	185	90
6	30	180	90
7	205	5	90
8	200	10	90
9	195	15	90
10	190	20	90
11	185	25	90
12	180	30	90

From Table 4.2, we can see that when the underlying between-subject variation is small (scenario 1-6), the Bayesian method with non-informative prior distributions has better confidence interval coverage of the true value of the subject random effect variance, $\sigma_s{}^2$, comparing to ML method. But when estimating the variance of subject-by-period random effect, $\sigma_p{}^2$, the advantage in the Bayesian method with non-informative prior distributions in terms of confidence interval coverage is not found, especially when the $\sigma_p{}^2$ is small (scenario 7-12).

From Table 4.3, we can conclude that the two methods nearly have the same type-1 error and power in the hypothesis testing conducted in the concentration-QTc model, which indicates when we set the non-informative prior distributions for the parameters in the Bayesian method, the Bayesian method and the ML method have similar ability in detecting QTc prolongation.

In conclusion, the Bayesian method with non-informative prior distributions on parameters perform similarly to the ML method in detecting a QTc prolongation effect in terms of similar power and type-1 error in the hypothesis testing conducted along with the concentration-QTc model. But when we are interested in estimating the random effect variance, the Bayesian method should be applied to produce a more reliable confidence interval for subject random effect variance, especially the when the between subject variance is small. But the ML method could provide a more reliable confidence interval for subject-by-period random effect variance.

Table 4.2 Coverage of variance component by confidence interval based on 1000 runs of simulations under each simulation scenario

Scenario	σ_s^2		σ_p^2	
	ML method	Bayesian method	ML method	Bayesian method
1	57.20%	99.70%	95.60%	95.90%
2	61.60%	99.00%	94.40%	94.20%
3	70.50%	95.80%	95.80%	95.50%
4	76.70%	94.20%	95.60%	95.50%
5	77.40%	87.30%	94.00%	94.40%
6	82.40%	86.60%	94.60%	94.30%
7	94.00%	95.50%	92.10%	84.50%
8	94.10%	95.40%	95.30%	89.40%
9	93.90%	95.00%	94.80%	92.60%
10	92.30%	93.90%	93.30%	92.10%
11	93.00%	94.40%	94.30%	94.10%
12	93.50%	94.80%	95.00%	94.50%

Table 4.3 Simulation results (type-1 error and power) in comparison between the Bayesian method with non-informative prior distribution and the ML method

True slope	Scenario	Bayesian method	ML method	True slope	Scenario	Bayesian method	ML method
0.33	1	69.80%	69.60%	1.67	1	6.20%	6.50%
	2	69.80%	69.80%		2	4.10%	4.10%
	3	70.60%	70.20%		3	5.60%	5.20%
	4	71.90%	71.20%		4	4.80%	4.60%
	5	71.40%	70.50%		5	4.70%	4.80%
	6	70.50%	70.10%		6	5.00%	5.00%
	7	95.10%	95.50%		7	5.80%	6.00%
	8	92.30%	92.50%		8	5.20%	5.70%
	9	89.60%	89.50%		9	4.80%	4.90%
	10	85.20%	85.30%		10	4.80%	4.50%
	11	84.30%	83.60%		11	4.40%	4.30%
	12	85.30%	85.10%		12	4.60%	4.80%

4.1.4 Comparison between the Bayesian method with informative prior distributions and the ML method in concentration-QTc model

An advantage of the Bayesian method over the ML method is that when prior information on parameter is available, the Bayesian method is able to incorporate the prior information in the model while the ML method cannot. For example, when we use concentration-QTc model with the Bayesian method to evaluate the QTc prolongation of biosimilar drug, we may use the information of the drug effect slope of its reference drug as prior distribution if it is available.

An explanatory simulation study is conducted to compare the Bayesian method and the ML method when reliable prior information is available for the drug effect slope.

Study design and QTc data simulation procedures are the same as those in Chapter 3. Only one simulation scenario is used with ${\sigma_s}^2 = 150$, ${\sigma_p}^2 = 60$, $\sigma^2 = 90$. Prior distribution of drug effect slope is assumed to be normal. When the true underlying β=0.33, the mean of the prior distribution of β has 4 scenarios, 3 of them are similar to the true underlying β, which are the same as the true underlying β, and around 10% smaller/larger than the true underlying β (0.33, 0.28, 0.38). The other scenario is when the prior information is not consistent with true underlying β from the trial (1.65). Similarly, when the true underlying β=1.65, the mean of the prior distribution of β also has 4 scenarios (1.65, 1.48, 1.82, 0.33). In addition, the standard deviation of the prior distribution of β has 2 scenarios (0.5, 1), because the standard deviation of the estimated drug effect slope in the previous simulation is around 0.5. In each simulation, similarly as before, the type-1 error and power in each scenario are also computed and compared between the two methods. The number of simulation for each scenario is 1000.

From Table 4.4, it can be concluded that when reliable prior information of drug effect slope is available, the Bayesian method always perform better than the ML method in terms of smaller type-1 errors and larger powers. But when the prior information is not consistent with the trial results, the Bayesian method may result in erroneous inference. And when the "belief" on prior distribution is stronger which is indicated by a smaller value of standard deviation of the prior distribution, the type-1 error gets larger and the power gets smaller which make the inference more erroneous.

Table 4.4 Simulation results (type-1 error and power) in comparison between the Bayesian method with informative prior distribution and the ML method

True slope	Prior distribution of β	Note	ML method	Bayesian method
0.33 (when the drug is "safe")	$N(0.33,\ 0.5^2)$	Prior agrees with trial	79.0%	99.9%
	$N(0.33,1)$		78.7%	88.6%
	$N(0.28,\ 0.5^2)$		77.5%	99.8%
	$N(0.28,1)$		77.9%	89.8%
	$N(0.38,\ 0.5^2)$		78.3%	99.4%
	$N(0.38,1)$		78.1%	88.7%
	$N(0.1.65,\ 0.5^2)$	Prior does NOT agree with trial	79.1%	48.8%
	$N(1.65,1)$		76.9%	70.2%
1.65 (when the drug is "NOT safe")	$N(1.65,\ 0.5^2)$	Prior agrees with trial	6.4%	2.8%
	$N(1.65,1)$		5.2%	3.5%
	$N(1.48,\ 0.5^2)$		5.9%	2.7%
	$N(1.48,1)$		5.9%	3.7%
	$N(1.82,\ 0.5^2)$		4.5%	0.5%
	$N(1.82,1)$		5.8%	2.8%
	$N(0.33,\ 0.5^2)$	Prior does NOT agree with trial	5.4%	67.9%
	$N(0.33,1)$		7.3%	14.1%

4.2 Generalized Estimating Equation (GEE)

4.2.1 Introduction to GEE

The generalized estimating equation (GEE) is a semiparametric method to estimate the parameters of a generalized linear model with correlated outcomes. It is proposed by Zeger and Liang (1986) and Zeger et al. (1988).

Let y_{kl} is the response for subject k at time l $(l = 1,2,3, \dots L)$, $\boldsymbol{y_k}$ is the $L \times 1$ vector consists of y_{kl}, $g(.)$ is a link function, $\boldsymbol{x_{kl}}$ is a $p \times 1$ vector of covariates for subject k at time l, $\boldsymbol{\theta}$ is a $p \times 1$ vector of regression coefficients. Furthermore, $\boldsymbol{V_k}$ is the covariance matrix of the within-subject outcomes $\boldsymbol{y_k}$. Given the marginal response $\mu_{kl} = E(y_{kl})$ to a linear combination of covariates $g(\mu_{kl}) = \boldsymbol{x_{kl}}'\boldsymbol{\theta}$, $\boldsymbol{\mu_k}$ is the $L \times 1$ vector consists of μ_{kl} . The GEE estimator of $\boldsymbol{\theta}$ is the solution of

$$\sum_{k=1}^{K} \frac{\partial \boldsymbol{\mu_k}}{\partial \boldsymbol{\theta}} \boldsymbol{V_k}^{-1} (\boldsymbol{Y_k} - \boldsymbol{\mu_k}) = \mathbf{0}$$

Typically, the equations can be solved via the Newton-Raphson algorithm. In the GEE framework, usually we have $var(y_{kl}) = \phi v(\mu_{kl})$, ϕ is a possibility unknown scale parameter, and $v(.)$ is a known function for the variance, for identity link function $g(\mu_{kl}) = \mu_{kl}$, we usually have $v(\mu_{kl}) = 1$. Then $\boldsymbol{V_k}$ usually has the form $\boldsymbol{V_k} = \phi \boldsymbol{A_k}^{1/2} \boldsymbol{R_k} \boldsymbol{A_k}^{1/2}$, where $\boldsymbol{A_k}$ is a $L \times L$ diagonal matrix with $var(y_{kl})$ as the l-th diagonal element, $\boldsymbol{R_k}$ is a $L \times L$ working correlation matrix which indicates the structure of $\boldsymbol{V_k}$. We can define the working correlation structure by ourselves, such as use the sample correlation matrix of the response vector, or we can also specify the structure of $\boldsymbol{V_k}$ and estimate the nuisance parameters associated with $\boldsymbol{V_k}$ together with $\boldsymbol{\theta}$ via iterative algorithm. Standard deviation of the GEE estimate of $\boldsymbol{\theta}$ is always given by Huber sandwich estimator (Freedman 2006).

GEE does not require the distributional assumptions, it only needs correct specification of the mean structure of the outcome given the covariates. When modeling correlated outcomes, it is a natural alternative of parametric models, such as ML method, especially when the ML estimate is hard to derive. GEE could provide consistent estimates of parameters and their associated standard errors even when the covariance structure is mis-specified, but the efficiency of the parameter estimate is higher when the covariance structure is closer to the true underlying covariance structure.

GEE is a popular method in the field of epidemiology when the outcomes are correlated and the population-averaged effects is of main interest (Hubbard et al. 2010), but the GEE is not able to estimate subject-level effect, which is the strength of the ML method in the mixed effects model. For example, in the concentration-QTc model, our assumption is that there is only a fixed effect associated with the drug effect *β,* which means the drug effect is a population-averaged effect, and it's same for all the subjects, then ML method and the GEE are both applicable to estimate *β.* If we assume the drug effect for subject k is β_k and further assume that β_k is distributed as $N(\beta, {\sigma_\beta}^2)$, then ML method is able to estimate β, ${\sigma_\beta}^2$ and each β_k, but GEE is only able to estimate β.

The main focus of the concentration-QTc model is the drug induced QTc prolongation in population level, which can be considered as population-averaged effect, ML method is widely used for this purpose but the GEE has not been applied to this yet. One of the pitfalls of the ML method is that there is still no definitive method for determining the covariance structure. Use of some model selection criteria, such as AIC, to pick up the covariance structure is one of the common methods, but Keselman et al. (1998) reported AIC, BIC only have less than 50 percent of chance to pick up the right covariance structure. Wrong specification of covariance matrix may result in erroneous inferences on parameter estimates. Since the GEE is consistent under the wrong specification of covariance structure of response vector when sample size is large, thus it is a natural alternative for the ML method when the covariance structure is unclear and the sample size is large relative to the number of measurements per subject. Therefore, the GEE is more applicable to a parallel SAD study, rather than a crossover SAD study.

4.2.2 Application of the GEE in concentration-QTc model

In Section 4.2.2, an application of the GEE in parallel SAD study design is proposed. A comparison between the GEE and the ML method when the covariance structure is wrongly specified is compared via simulation study in Section 4.4.

An example of parallel SAD study design is as follows. In parallel SAD design, number of groups is equal to the number of doses, in each dose group, we usually have 8 subjects, 6 of them have a dose of tested drug and the other 2 take placebo. The ECG measurements and drug concentration measurements are collected in the same time points in a 24 hour post-dose time interval.

Let y_{ks} be the QTc value for subject k ($k = 1,2, \dots, K$) at time t_s ($s = 1,2,3, \dots, T$), and C_{ks} be the drug concentration for subject k at time t_s. In parallel SAD design, each subject is only measured in a single period, thus here we only consider the subject random effect to account for the within-subject correlation. So the rQTc model in the parallel design is formulated as

$$y_{ks} = \beta * C_{ks} + T_s + b_k + \epsilon_{ks},$$

here β is the slope of drug effect, T_s is the categorical time effect of time t_s to account for the circadian rhythm effect, b_k is the subject random effect and we usually assume $b_k \sim N(0, {\sigma_s}^2)$, ϵ_{kt} is the random error and we usually assume $\epsilon_{ks} \sim N(0, \sigma^2)$.

Usually, we use the ML method to estimate the parameters. And the normality assumptions on subject random effect b_k and random error ϵ_{ks} are essential for the ML method, but no distributional assumption is required for the GEE.

Let $\boldsymbol{X_k}$ denote the design matrix contains the data of drug concentration and categorical time effect for subject k, and $\boldsymbol{\varphi} = (\beta, T_1, T_2, \dots, T_T)$ denote the parameters to be estimated, $\boldsymbol{V_k} = COV(\boldsymbol{y_k})$ is the covariance matrix of $\boldsymbol{y_k}$, it's compound symmetry structure if the model assumption holds. Then the rQTc model can be reformulated as

$$\boldsymbol{y_k} = \boldsymbol{X_k\varphi} + \boldsymbol{b_k} + \boldsymbol{\epsilon_k}.$$

The log-likelihood function of the model is

$$\boldsymbol{l(\varphi)} = \frac{\boldsymbol{K}}{\boldsymbol{2}}\boldsymbol{log(2\pi)} - \frac{\boldsymbol{1}}{\boldsymbol{2}}\boldsymbol{\Sigma_{k=1}^{K}}\left[\boldsymbol{log}(|\boldsymbol{V_k}|) + (\boldsymbol{y_k} - \boldsymbol{X_k\varphi})^{\boldsymbol{T}}\boldsymbol{V_k^{-1}}(\boldsymbol{y_k} - \boldsymbol{X_k\varphi})\right]$$

To solve $\boldsymbol{\varphi}$, we have $\frac{\partial \boldsymbol{l(\varphi)}}{\partial \boldsymbol{\varphi}} = 0$, such that $\sum_{\boldsymbol{k=1}}^{\boldsymbol{K}} \boldsymbol{X_k}\,{\boldsymbol{V_k}}^{\boldsymbol{-1}}(\boldsymbol{y_k} - \boldsymbol{X_k\varphi}) = \boldsymbol{0}$.

When applying the GEE to concentration-QTc model, the link function is linear, thus $\boldsymbol{\mu_k} = \beta * \boldsymbol{C_k} + \boldsymbol{T} = \boldsymbol{X_k\varphi}$, $\boldsymbol{V_k} = COV(\boldsymbol{y_k})$ is the covariance matrix of $\boldsymbol{y_k}$ with a compound symmetry structure under our model assumption. So the estimating equation of the GEE to estimate $\boldsymbol{\varphi}$ is as follows,

$$\sum_{\boldsymbol{k=1}}^{\boldsymbol{K}} \boldsymbol{X_k}\,{\boldsymbol{V_k}}^{\boldsymbol{-1}}(\boldsymbol{y_k} - \boldsymbol{X_k\varphi}) = \boldsymbol{0}$$

The Newton-Raphson method is always used to estimate $\boldsymbol{\varphi}$ from the above equation (Yan and Fine 2002, Yan 2004). When $\boldsymbol{V_k}$ is considered a nuisance parameter when estimating $\boldsymbol{\varphi}$, we can specify a structure of the working correlation matrix, such as compound symmetry, AR(1), and estimate $\boldsymbol{\varphi}$ and nuisance parameters associated with the working correlation matrix using some iterative algorithm. We can also give a fixed correlation matrix as the working correlation

matrix, such as the sample correlation matrix, since if the true underlying correlation structure of $\boldsymbol{y_k}$ is unknown, sample correlation matrix should serve as a reasonable approximation to it.

We note that the equation in the GEE is the same as the equation in the ML method, therefore when the covariance structure is the same for the two methods, the parameter estimates should be similar between the two methods. For example, if we have only random subject effect in the ML method which means the covariance matrix structure of the response vector in the ML method is compound symmetry, and when using the GEE, we also specify the working correlation structure to be compound symmetry, the parameter estimates from the two methods should be very close. In general, when the link function in the GEE is linear, and the covariance matrix structure of the response vector is the same for the GEE and the ML method, the parameter estimates should be very close between these two methods.

4.3 Generalized Method of Moments (GMM)

4.3.1 Introduction to GMM

Generalized method of moments (GMM) is a method for estimating parameters in statistical models. It was developed by Hansen in (1982), Hansen shared the 2013 Nobel Prize in Economics for this work.

GMM can be seen as a generalization of many other estimation methods, such as the ordinary least square (OLS), the generalized estimating equation (GEE) and the maximum likelihood (ML) method. Certain number of moment conditions need to be specified and these moment conditions are functions of the model parameters and the data, such that the expectation of the function is zero at the underlying true value of the parameters. Then the GMM method estimates the parameters by minimizing certain norm of the sample averages of the moment conditions. The requirement of the GMM is that we have some appropriate moment conditions available to estimate the parameters, no distributional assumption on the data is required.

Suppose we have available data consists of K observation vectors $\{\boldsymbol{Y_k}\}, k = 1,2, \dots, K$, each observation $\boldsymbol{Y_k}$ is a n-dimension multivariate random vector. In addition, $\boldsymbol{\theta}$ is a p-dimensional parameter of interest, $\boldsymbol{\theta}_0$ is the underlying true value of $\boldsymbol{\theta}$. In order to apply the

GMM, we need to have some moment conditions $f(\boldsymbol{Y}, \boldsymbol{\theta})$, which is a function of the data and the parameter, such that

$$E[f(\boldsymbol{Y_k}, \boldsymbol{\theta}_0)] = \mathbf{0}.$$

Then we will have the sample average of the moment conditions

$$m(\boldsymbol{\theta}) = \frac{\mathbf{1}}{\boldsymbol{K}} \sum_{\boldsymbol{k=1}}^{\boldsymbol{K}} f(\boldsymbol{Y_k}, \boldsymbol{\theta}),$$

the GMM estimator $\widehat{\boldsymbol{\theta}}$ can solved by setting the sample moment condition equal to 0,

$$m(\boldsymbol{\theta}) = \frac{\mathbf{1}}{\boldsymbol{K}} \sum_{\boldsymbol{k=1}}^{\boldsymbol{K}} f(\boldsymbol{Y_k}, \boldsymbol{\theta}) = 0.$$

When the number of moment conditions is larger than the number of parameters to estimate, Hansen (1982) introduced a positive definite weight matrix $\boldsymbol{W}$, and the GMM estimator $\widehat{\boldsymbol{\theta}}$ can be written as

$$\widehat{\boldsymbol{\theta}} = \arg min_{\boldsymbol{\theta}} \left(\frac{\mathbf{1}}{\boldsymbol{K}} \sum_{\boldsymbol{k=1}}^{\boldsymbol{K}} f(\boldsymbol{Y_k}, \boldsymbol{\theta})\right)' \boldsymbol{W} \left(\frac{\mathbf{1}}{\boldsymbol{K}} \sum_{\boldsymbol{k=1}}^{\boldsymbol{K}} f(\boldsymbol{Y_k}, \boldsymbol{\theta})\right).$$

The GMM estimator solves the estimating equation

$$\left(\frac{\partial m(\boldsymbol{\theta})}{\partial \boldsymbol{\theta}}\right)' \boldsymbol{W}\, m(\boldsymbol{\theta}) = \mathbf{0}.$$

$\frac{\partial m(\boldsymbol{\theta})}{\partial \boldsymbol{\theta}}$ and $\boldsymbol{W}$ together gives the relative importance of each of the original moment conditions used in the GMM (Lai and Small 2007). The "two-step" approach proposed by Hansen is one of the most popular one in deriving the GMM estimator.

4.3.2 Application of GMM in concentration-QTc model

In this section, the application of the GMM to concentration-QTc model is introduced.

Similar to the GEE, the GMM also needs a large sample size thus it is more suitable for a parallel SAD design. We will revisit the model for the parallel SAD design,

$$y_{ks} = \beta * C_{ks} + T_s + b_k + \epsilon_{ks}.$$

By rewriting the model we have

$$\boldsymbol{y_k} = \boldsymbol{X_k}\boldsymbol{\varphi} + \boldsymbol{b_k} + \boldsymbol{\epsilon_k}.$$

Here $\boldsymbol{X_k}$ denote the design matrix containing the data of drug concentration and categorical time effect for subject k, $\boldsymbol{\varphi}$ is the vector of parameters including β and T_s, and $\boldsymbol{V_k} = COV(\boldsymbol{y_k})$ is the covariance matrix of $\boldsymbol{y_k}$.

We have $E\left[\boldsymbol{X_k}\boldsymbol{V_k}^{-1}(\boldsymbol{y_k} - \boldsymbol{X_k}\boldsymbol{\varphi})\right] = \boldsymbol{0}$, then the moment condition in this case is $m(\boldsymbol{\varphi}) = \frac{\boldsymbol{1}}{\boldsymbol{K}}\sum_k \boldsymbol{X_k}\boldsymbol{V_k}^{-1}(\boldsymbol{y_k} - \boldsymbol{X_k}\boldsymbol{\varphi})$.

To obtain the GMM estimates, we need to solve the equation $m(\boldsymbol{\varphi}) = \boldsymbol{0}$. By using a fixed covariance matrix for $\boldsymbol{V_k}$, GMM is equivalent to GEE with fixed working correlation matrix in terms of parameter estimate. If $\boldsymbol{V_k}$ is a diagonal matrix with the same elements on the diagonal, then GMM is equivalent to GEE with independent working correlation structure. However, when GEE with an estimated working correlation matrix, such as a compound symmetry matrix, that has nuisance parameters other than $\boldsymbol{\varphi}$, then GEE cannot be embedded into the framework of the GMM (Ziegler 2011).

4.4 Comparison between the ML method, GEE, GMM in concentration-QTc model

4.4.1 Simulation study in comparing the ML method, GEE and GMM

In Sections 4.2 and 4.3, we proposed novel applications of the GEE and the GMM in concentration-QTc model and connections between them. In Section 4.4, simulation study is conducted to compare the ML method, the GEE and the GMM in concentration-QTc model.

In Section 4.2.2, we gave a brief introduction to parallel SAD design which is revisited here.

Table 4.5 Study design of SAD parallel study

Period	Group	Sample Size	Time of measurements
1	DOSE1	"3+1"	-1, 1, 2, 4, 6, 8, 12, 15, 24
2	DOSE2		
3	DOSE3		
4	DOSE4		
5	DOSE5		
6	DOSE6		
7	DOSE7		
8	DOSE8		

An example of parallel SAD study is shown in Table 4.5, each subject only has one dose of drug or placebo, number of subjects in each sequence usually follows a "3:1" ratio which means the number of subjects taking drug in each period is as three times as many as those taking placebo. The dosages 10mg, 50mg, 100mg, 150mg, 200mg, 300mg, 400mg, 600mg are assigned to dose1-dose8 as we did in Chapter 3. For each subject, one QTc measurement is taken before the drug/placebo administration, and 8 post-dose QTc measurements and drug concentrations are taken at the 1st, 2nd, 4th, 6th, 8th, 12th, 15th, and the 24th hour post-dose.

To generate the repeated measures QTc data, we assume QTc variation is only subject to subject random effect, which is normally distributed with mean 0, variance ${\sigma_s}^2$ and random error is also normally distributed with mean 0, with variance σ^2. We fix ${\sigma_s}^2 = 180$ and $\sigma^2 = 90$. AR(1) within-day random error is simulated with 9 correlations, $\rho = 0.1, 0.2, \dots, 0.8, 0.9$, in these AR(1) within-day random error scenarios, the within-subject covariance is calculated as $\left({\sigma_s}^2 + {\sigma_p}^2\right) * \boldsymbol{J}_{(T+1)} + \sigma^2 * \boldsymbol{\rho}_{(\boldsymbol{T+1})*(\boldsymbol{T+1})}$, where the (s, s')th element of $\boldsymbol{\rho}_{(\boldsymbol{T+1})*(\boldsymbol{T+1})}$ is $\rho^{|t_s - t_{s'}|}$, here t_s denotes the time corresponding to the s-th time point in each period. Drug concentration simulation process is the same as that in Chapter 3 using a one-compartmental model.

Since the GEE and the GMM both need large sample size to obtain reasonable parameter estimates, we use the sample size scheme of "6+2" (total sample size of 64) in each scenario at first and then increase the sample size for the purpose to investigate the properties of each method.

Five methods are included for comparison, the ML method with subject random intercept and independent random error, the GEE with fixed sample correlation, the GEE with compound symmetry correlation structure, the GEE with independent correlation structure, and the GMM with moment condition $m(\boldsymbol{\varphi}) = \frac{\mathbf{1}}{\boldsymbol{K}}\sum_{\boldsymbol{k}} \boldsymbol{X_k}(\boldsymbol{y_k} - \boldsymbol{X_k}\boldsymbol{\varphi})$ as described in Section 4.3.2. Pepe and Anderson (1994) and Pan et al. (2000) mentioned that a diagonal working covariance matrix should be used or a key assumption should be verified, especially for time-varying covariates. Since drug concentration is a time-varying covariate, the GEE with independent correlation structure and its counterpart in GMM are included here. By comparing the 5 methods in AR(1) random error structure, we aim to investigate performances of each method when the covariance matrix structure is wrongly specified for ML method under different sample size scenario.

For each scenario, type-1 error and power in detecting a "safe" drug in the hypothesis testing conducted in concentration-QTc model is calculated. Bias and MSE in estimating β are also compared. The number of simulation is 1000 under each simulation scenario.

4.4.2 Simulation results in comparing the ML method, GEE and GMM

From Figure 4.1, we can see that in all the sample size scheme, the type-1 error of the ML method increases when the covariance matrix structure is wrongly specified for ML method (ρ>0). Type-1 errors of all the GEE methods and the GMM are similar and consistent with change of the AR(1) correlation, and type-1 errors tend to decrease as sample size increases. This indicates that in terms of type-1 error, the ML method is more sensitive to mis-specification of the covariance matrix structure while the GEE methods and the GMM are more robust.

From Figure 4.2, the GEE with fixed sample correlation has a comparable power to the ML method in detecting a "safe" drug in all the simulation scenarios; however considering the ML method has inflated type-1 error when the covariance matrix structure is mis-specified (ρ > 0), we conclude that in terms of hypothesis testing in concentration-QTc model, the GEE with fixed sample correlation is superior to the ML method. The power of the GEE with compound symmetry correlation structure tends to decrease when the AR(1) correlation increases when the sample size is not that large ("6+2", "9+3"), however it has comparable power to GEE with fixed

sample correlation and the ML method when the sample size is large ("36+12", "72 +24"). This should be due to that the compound symmetry correlation structure is more severely violated when AR(1) correlation becomes larger which may reduce the efficiency of the GEE with compound symmetry structure. The GEE with independent correlation structure and the GMM only have comparable power to the other methods when the sample size is large ("36+12", "72+24"), which indicates that the choice of correlation structure for the GEE, and the choice of moment condition for the GMM would affect the efficiency of the parameter estimates.

Figure 4.3 indicates that the bias of the estimated drug effect $\hat{\beta}$ of all the models are similar when the slope is small (β=0.33). GEE with sample correlation has larger bias when the drug effect slope is large (β=1.65). However Figure 4.4 indicates that GEE with fixed sample correlation as working correlation matrix always has comparable MSE in estimating β to the ML method when the covariance matrix structure is correctly specified for the ML method ($\rho=0$), while the GEE with fixed sample correlation has smaller MSE than the ML method when the covariance matrix structure is wrongly specified for the ML method ($\rho > 0$). The MSE of the GEE with independent structure and the GMM are mostly affected by the sample size, this indicates that more careful choice of working correlation matrix structure in the GEE and moment condition in the GMM would provide a more efficient parameter estimates, although their estimates are still consistent.

The performance of parameter estimates (bias, MSE) between the ML method and the GEE with compound symmetry working correlation matrix structure are very similar, so does the performance between the GEE with independent working correlation structure and the GMM. These results further confirm the connections between these methods as illustrated in Sections 4.2 and 4.3.

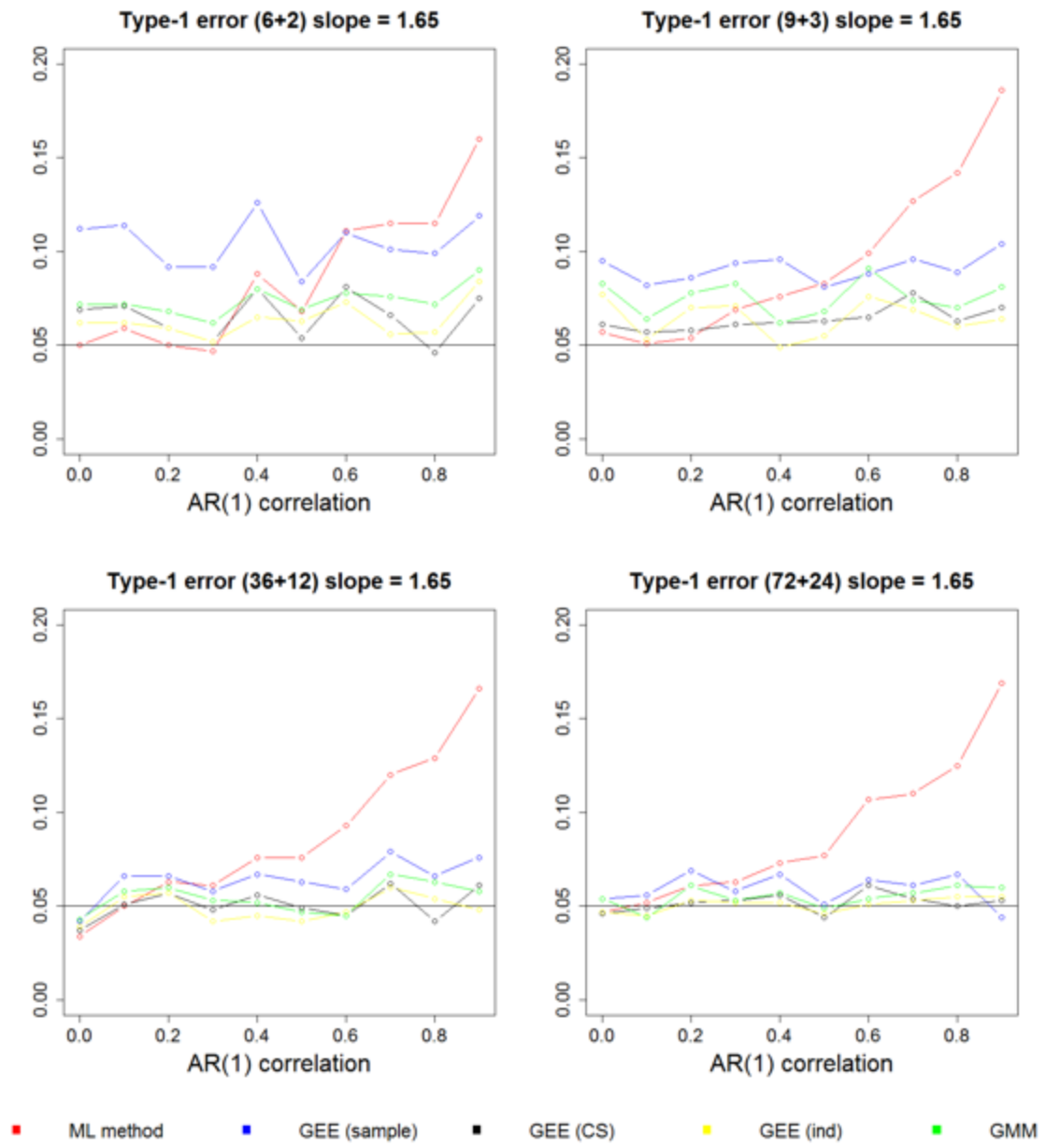

Figure 4.1 Simulation results (type-1 error) in comparing the ML method, the GEE and the GMM

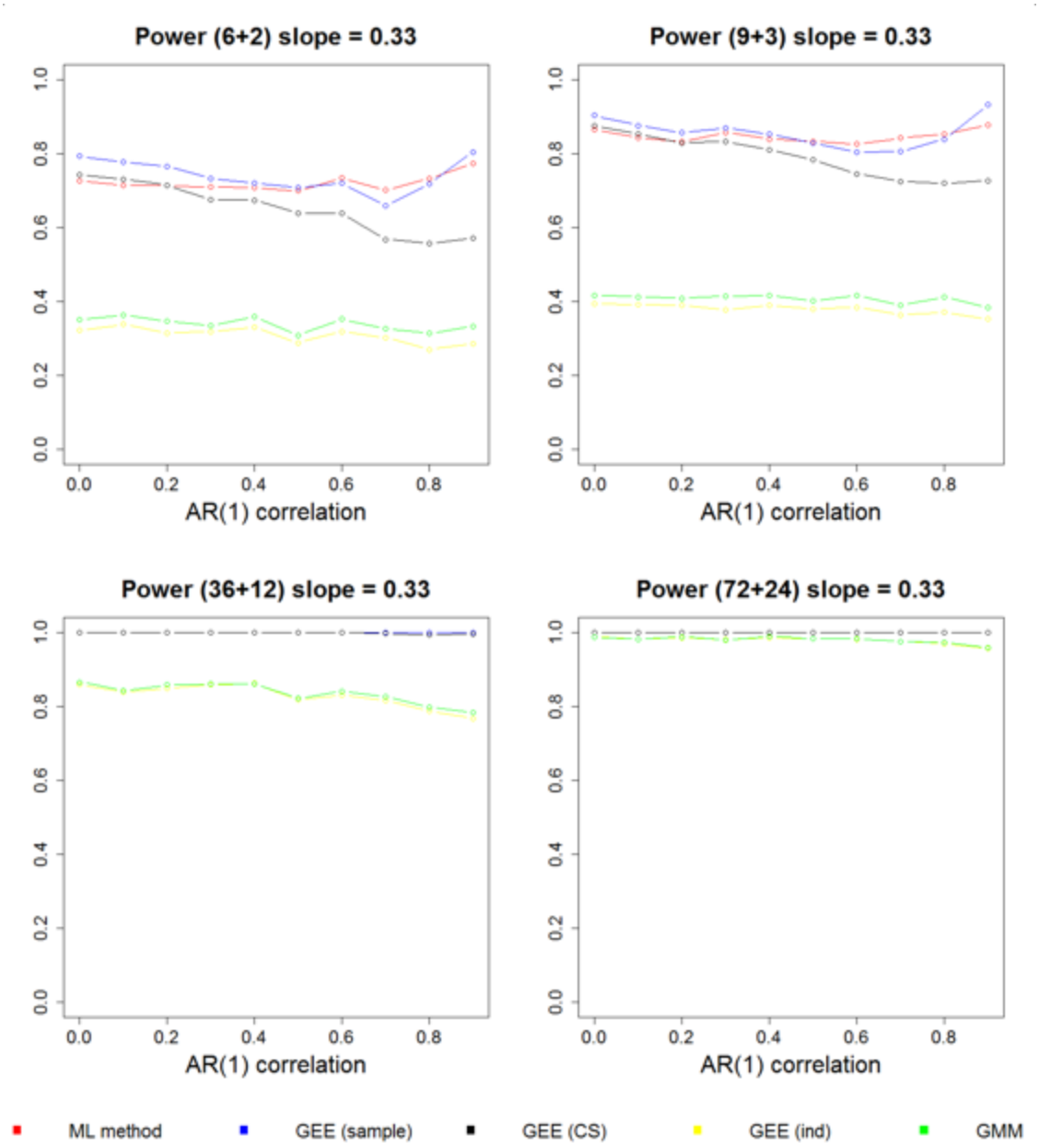

Figure 4.2 Simulation results (power) in comparing the ML method, the GEE and the GMM

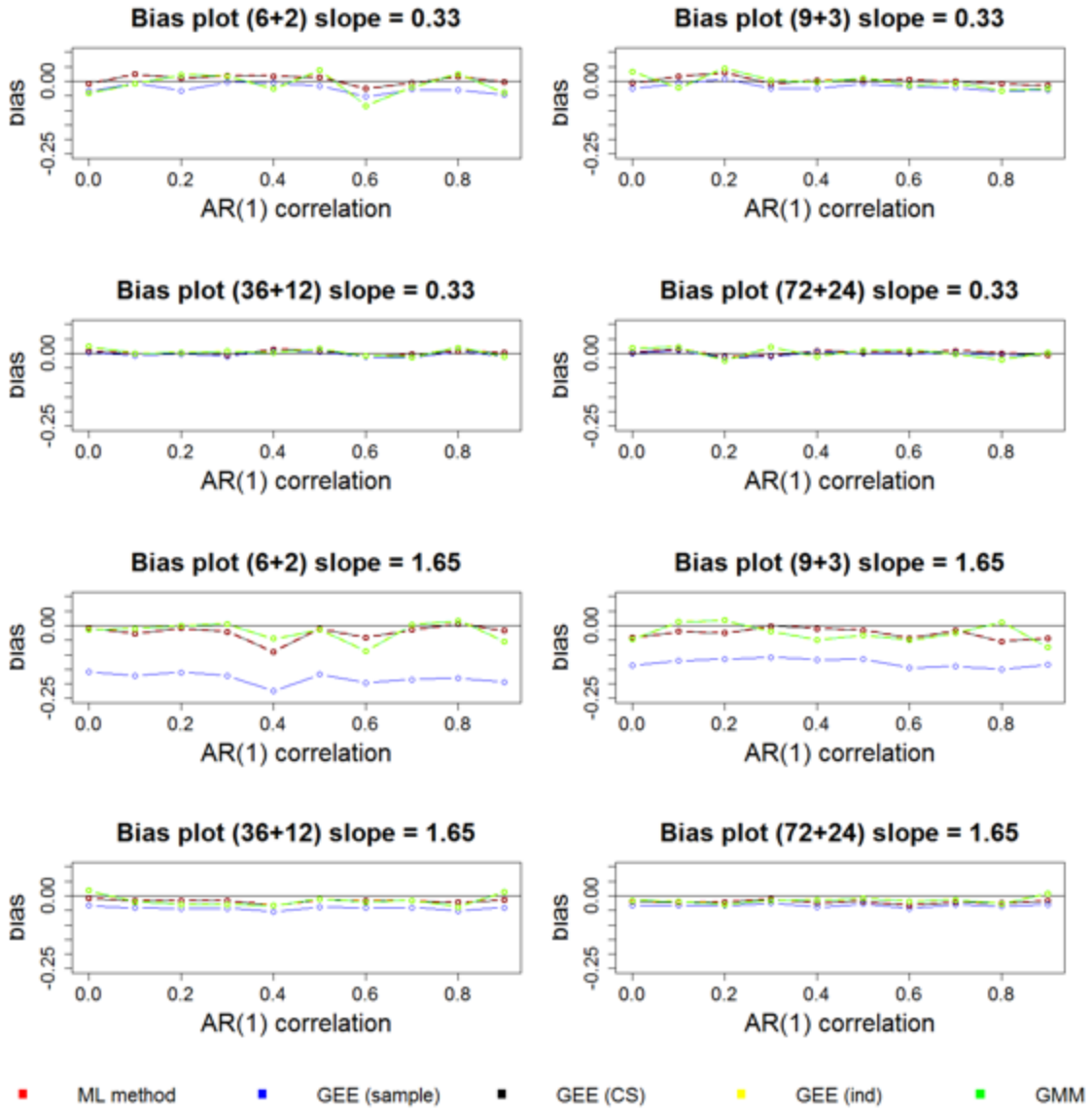

Figure 4.3 Bias of each method when estimate β (calculated as the mean difference between estimated $\hat{\beta}$ and true β)

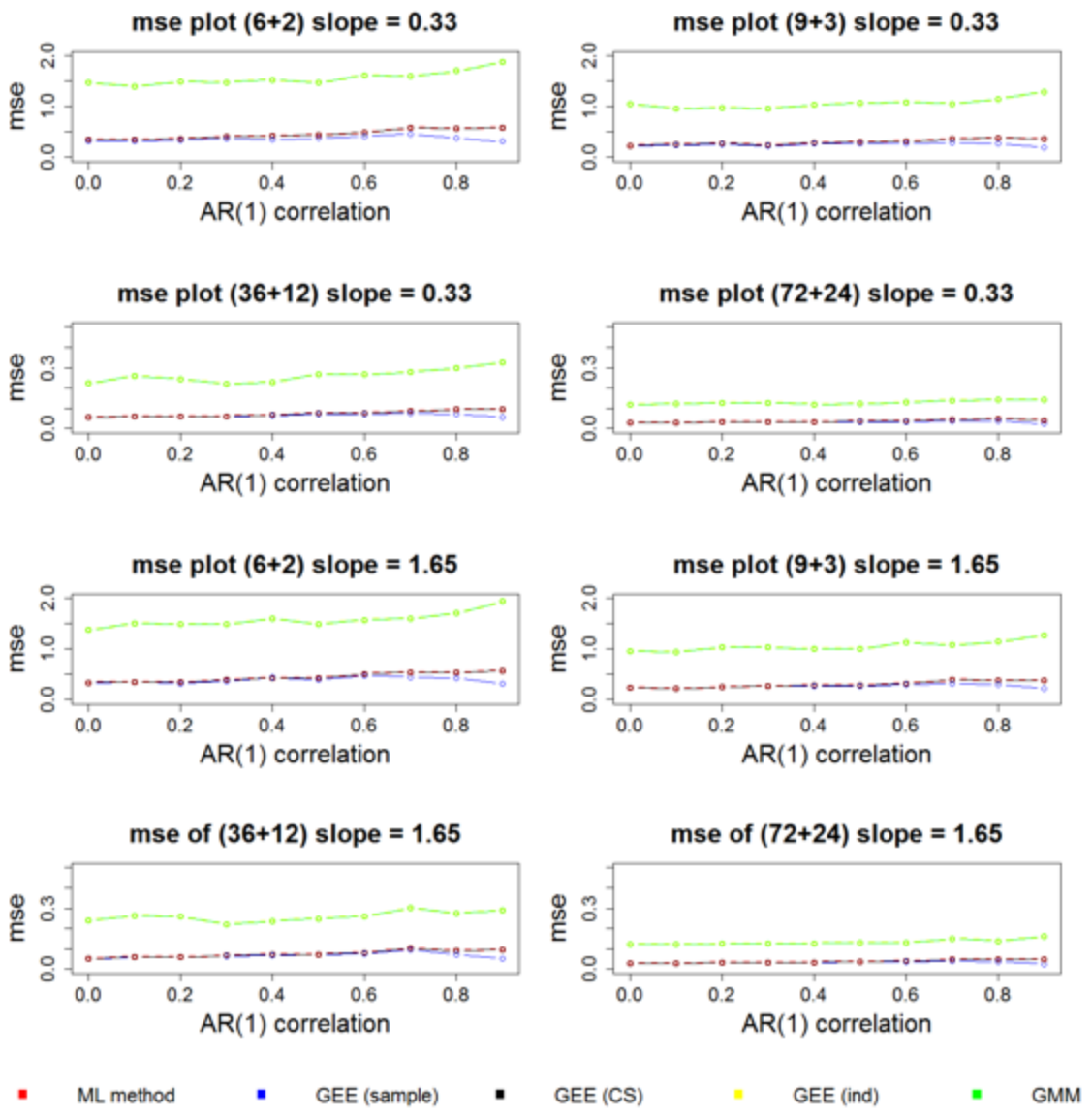

Figure 4.4 MSE of each method when estimate β (calculated as the mean squared difference between $\hat{\beta}$ and true β)

4.5 Conclusion and discussion

In Chapter 4, several novel applications in the concentration-QTc model are proposed, the comparisons between the traditional ML method and the newly proposed methods are also conducted. Comparison between these methods are summarized in Table 4.6 and Table 4.7.

For mixed effects model, the Bayesian method has the ability to include prior distribution of the parameters while the ML method cannot. With reliable prior distributions on the parameters, the Bayesian method outperforms ML method with larger power and smaller type-1 error in the hypothesis testing conducted in concentration-QTc model. But inferences may be erroneous when the prior information is not consistent with the trial result. Thus application of the Bayesian method with informative prior information should be cautious when the parameter estimate is quite different from the prior distribution. When we don't have a specific prior distributions for parameters, the Bayesian method with non-informative prior distribution has similar performances to the ML method in terms of similar power and type-1 error in detecting a "safe" drug.

The GEE and the GMM are two popular methods in modeling correlated outcomes. Their distribution free nature render these two methods more attractive when the maximum likelihood function is hard to derive. Furthermore, when the covariance structure is wrongly specified, which is always a case in mixed effects model with the ML method, the GEE and the GMM can still provide consistent parameter estimation when the sample size is relatively large. The GEE method with a sample correlation matrix as the working correlation matrix has comparable power to the ML method in excluding a small QTc prolongation effect and identifying a "safe" drug, and it can better control the type-1 error while the type-1 error of the ML method is severely affected by the wrong specification of covariance matrix. Moreover, the GEE with sample correlation can also provide parameter estimates with smaller mean square error comparing to ML method.

Table 4.6 Summary of comparison between the ML method and the Bayesian method

	ML method	Bayesian method
How to treat parameter	1. Treat parameter as a single point 2. Not able to consider prior belief	1. Treat parameter as a random variable 2. Able to include prior belief in the model if available
Parameter estimate	Obtain point estimate of the parameter by maximizing the likelihood function	Obtain a posterior distribution of the parameter and make inference of parameter based on it (e.g. use maximum a posteriori probability (MAP) estimate to get a point estimate)
Assumption and performance	1. Both need correctly specify the distribution of the data give the parameters 2. In the mixed effects model, the Bayesian method with non-informative prior distributions yield more confidence interval coverage on the subject random effect variance 3. Comparing to ML method, the Bayesian method yield more efficient parameter estimate with reliable prior information, but inference may be erroneous when the prior information is not consistent with the data	

Table 4.7 Summary of comparison between the ML method, the GEE and the GMM

	ML method	GEE	GMM
Focus of interest	Both population-level effect and subject-level effect	Population-level effect	Depends on the moment condition
Assumption	Correctly specified distribution and covariance structure for the data	Correctly specified mean structure	Correctly specified moment condition
Connection	1. The ML method and the GEE are similar when the link function in GEE is linear and covariance structure is specified the same for the methods 2. The ML method is in the GMM framework as we specified the first derivative of the likelihood function as the moment condition 3. The GEE with independent correlation structure and the GEE with fixed correlation matrix are both in the framework of the GMM		

Chapter 5 Real data analysis

Data analysis on real data from a phase 1 SAD data is presented in this chapter. Models with the ML method (rQTc, ΔQTc, ΔΔQTc and cΔQTc), the Bayesian method, the GEE and the GMM are all compared in the real data analysis.

5.1 Introduction to the real data

The data is from a typical 4-period phase 1 single ascending dose (SAD) study with 16 subjects, each subject have 3 periods of drug with different doses and 1 period of placebo, the study design is summarized in Table 5.1.

Table 5.1 Study design of real data from a phase 1 crossover SAD study

Number of Subjects	Period 1	Period 2	Period 3	Period 4
N=2	Placebo	DOSE3	DOSE5	DOSE7
N=2	DOSE1	Placebo	DOSE5	DOSE7
N=2	DOSE1	DOSE3	Placebo	DOSE7
N=2	DOSE1	DOSE3	DOSE5	Placebo
N=2	Placebo	DOSE4	DOSE6	DOSE8
N=2	DOSE2	Placebo	DOSE6	DOSE8
N=2	DOSE2	DOSE4	Placebo	200mg
N=2	DOSE2	DOSE4	DOSE6	DOSE8

Information of dosage is not revealed here, but the order of dosage is essentially the same as Table 1.1 with DOSE1<DOSE2<DOSE3<DOSE4<DOSE5<DOSE6<DOSE7<DOSE8. In each period, each subject has two pre-dose ECG measurements, thus the average of the two QTc values is considered as the period-specific pre-dose QTc baseline. In each period, 7 drug

concentration measurements and QTc measurements are taken at the 1st, 2nd, 4th, 6th, 8th, 12th and 24th hour post-dose. We choose the highest dose (DOSE8) as the dose of interest.

5.2 Analysis of the real data

5.2.1 Comparison of model efficiencies

First, rQTc, ΔQTc, ΔΔQTc and cΔQTc models are fitted using the classic ML method, respectively and the results of the estimated drug effect are summarized in Table 5.2.

Table 5.2 Estimated QTc prolongation from rQTc, ΔQTc, ΔΔQTc and cΔQTc models

Model	Estimated drug effect slope $\hat{\beta}$	Standard error of estimated drug effect slope $\hat{\beta}$	1-sided 95% upper bound of $\hat{\beta}$	1-sided 95% upper bound of QTc prolongation
rQTc	0.00407	0.00515	0.0125	6.51ms
ΔQTc	0.00295	0.00538	0.0118	6.15ms
cΔQTc	0.00488	0.00452	0.0123	6.41ms
ΔΔQTc	0.00949	0.00447	0.0168	8.75ms

From Table 5.2, we can see that the standard error is smaller in the cΔQTc model comparing to the rQTc model and the ΔQTc model, the estimated drug effect slope and the estimated upper bound of the QTc prolongation are similar between the 3 models.

The estimated variance of drug effect slope is the smallest in the ΔΔQTc model, but the drug effect slope estimate of the ΔΔQTc model is quite different from the other 3 models, which makes it produce the largest upper bound of the QTc prolongation, this may be due to several reasons, such as the true underlying unknown covariance structure of repeated measures QTc data is not exactly double compound symmetry. But based on the fact that the ΔΔQTc model gives the largest value of upper bound of the estimated QTc prolongation, and this estimate is quite different from the other 3 models, this may indicate that the ΔΔQTc model should be used with caution even if it has the smallest standard error of estimated drug effect slope.

5.2.2 Application of the Bayesian method, GEE and GMM

First, we fit the rQTc model with both the ML method and the Bayesian method with non-informative prior distribution. For the Bayesian method, the estimated drug effect slope is obtained by calculating the mean from the posterior sample, and the posterior median (0.00415) is similar to the mean. Standard error of estimated drug effect is also calculated from the posterior sample of drug effect β. The 1-sided 95% upper bound of $\hat{\beta}$ is computed as the 95th percentile of the posterior sample. From Table 5.3, we can conclude that the ML method and the Bayesian method produce very similar estimates. Table 5.4 shows that estimated standard deviation with its two-sided 95% confidence interval based on the ML method and the Bayesian method. Both methods produce similar results in estimating the variance component for rQTc model.

Moreover, the GEE and the GMM are also fitted here although they are more suitable for a parallel design when the sample size is relatively large. From Table 5.3, the GEE with different working correlation matrix structures produce different estimates of drug effect slope $\hat{\beta}$, and this may be a result of the small sample size of the crossover SAD study. Although the upper bound of QTc prolongation from the GEE with CS correlation structure, the GEE with independent correlation structure and the GMM are all less than 10ms, the significantly inconsistent estimates between the GEE with different correlation structures indicate that it should be cautious when apply the GEE with data from crossover SAD study, when the sample size is relatively small compared to the number of measurements per subject.

Table 5.3 Estimated QTc prolongation from the ML method, the Bayesian method, the GEE with sample correlation, the GEE with compound symmetry structure, the GEE with independent structure and the GMM

Model	Estimated drug effect slope $\hat{\beta}$	Standard error of estimated drug effect slope $\hat{\beta}$	1-sided 95% upper bound of $\hat{\beta}$	1-sided 95% upper bound of QTc prolongation
ML method	0.00407	0.00515	0.0125	6.51ms
Bayesian method	0.00407	0.00512	0.0126	6.57ms
GEE (sample correlation)	0.0433	0.0368	0.104	54.2ms
GEE (CS)	0.00463	0.00349	0.0104	5.42ms
GEE (Independent)	-0.00291	0.00612	0.00716	3.73ms
GMM	-0.00291	0.00838	0.0109	5.68ms

Table 5.4 Estimated standard deviation and two-sided 95% confidence interval of random error, subject random effect, subject-by-period random effect between the ML method and the Bayesian method for the rQTc model

Method	σ standard deviation of random error	σ_s standard deviation of subject random effect	σ_p standard deviation of subject-by-period random effect
ML method	11.20 (10.36, 11.94)	12.41 (8.36, 18.21)	6.43 (4.76, 8.54)
Bayesian method	11.20 (10.5, 12.1)	12.49 (8.66, 19.40)	6.44 (4.75, 8.57)

Chapter 6 Conclusion and future work

6.1 Conclusion and contribution

Concentration-QTc model is becoming increasingly popular in evaluation of proarrhythmic risk in drug application. However several outstanding statistical issues within the current development of concentration-QTc model must be addressed. This dissertation endeavors to improve the current concentration-QTc model from the following aspects.

First, when researchers model the circadian rhythm effect of QTc with multi-oscillator function, limitations in sampling strategy to identify the true underlying multi-oscillator function is never addressed. We conduct simulation studies to evaluate the ability of the current sampling strategy in identifying the true underlying function. The result is not surprising that due to the limited number of QTc measurements between the 12^{th} hour and the 24^{th} hour post-dose, unevenly distributed sampling strategy produces unstable parameter estimates and thus we are not able to pick up the right multi-oscillator function. To further validate our hypothesis that the poor identifiable rate of the current sampling strategy is due to the unevenly spaced sampling strategy, we revised the sampling strategy to make the sampling time points more evenly distributed. The identifiable rate to the true model has thence been significantly increased. Therefore, thorough considerations are needed when using multi-oscillator functions when the time points are limited and also relatively unevenly distributed. In addition, we found the usage of categorical time covariate is another reasonable alternative (Huh and Hutmacher 2015) to model the circadian rhythm effect in the concentration-QTc model.

Secondly, when conducting concentration-QTc model with data from phase 1 crossover single ascending dose (SAD) study with period-specific QTc baseline measurement, different concentration-QTc models have different ways to treat the period-specific pre-dose QTc baseline. The comparison of efficiency between each model has not been discussed. We compare the efficiencies of each model under the same framework by assuming the covariance structure of the data is double compound symmetry. We proposed a cΔQTc model that reduces the variance

of estimated drug effect slope by including period-specific pre-dose QTc baseline and subject-averaged pre-dose QTc baseline as covariates in the model. By deriving and comparing the variance of the estimated drug effect slope $\hat{\beta}$ for each model we can conclude that our proposed cΔQTc model is most efficient comparing to the existing models. Simulation studies also validated the advantage in efficiency of our proposed model.

Finally, methods other than the maximum likelihood (ML) method are rarely applied in concentration-QTc model. We propose 3 novel applications of statistical methods here. First, we provide a framework of Bayesian method and shows that the Bayesian method with non-informative prior distributions has advantage over ML method in estimating the variance of subject random effect variance, especially when the variance of subject random effect is small. Also, the Bayesian method with reliable prior distributions outperforms the ML method with larger power and smaller type-1 error in the hypothesis testing conducted with concentration-QTc model. Second, we discuss the connections between the ML method, the generalized estimating equation (GEE) and the generalized method of moments (GMM). We also applied the GEE and the GMM to concentration-QTc model. Simulation study shows that GEE with fixed sample correlation has comparable performance as the ML method in terms of power to detect a "safe" drug while it can better control the type-1 error rate. The GEE with sample correlation always produces drug effect slope estimate with smaller mean square error when the covariance matrix specification of the ML method is incorrect ($\rho > 0$).

6.2 Future work

First, the advantage of the proposed cΔQTc model is based on the fact that the covariance matrix structure of repeated measures QTc data is double symmetry, whether the advantage in efficiency of the cΔQTc model still holds in cases other than double symmetry covariance matrix structure, such as unspecified structure, TOEP structure, is not clear. We will explore the properties of our proposed model in more covariance matrix structures.

Second, we provide a framework of applying the GMM in rQTc model with a certain moment condition and show the GMM could provide consistent estimate of drug effect slope,

although it is not efficient. Some other moment conditions may be able to achieve a reasonable GMM estimate with a smaller size comparing to the current moment condition we used. Further work will also be done in exploring a better choice of moment condition when applying the GMM method.

Last but not the least, all of our work are based on the current study design of phase 1 single ascending dose (SAD) studies, how to improve the reliability of QTc prolongation estimate with concentration-QTc model by modifying SAD study design is not clear. Thus more work will also be done in investigating the improvement of SAD study design to make the QTc prolongation estimate from SAD study to be more reliable. The most immediate study maybe to examine the possibility of increased sample size in SAD study and its benefit in terms of test power and the type I error rate. Given that the SAD study with a very modest sample size is proposed to replace the large TQT study in assessing the proarrhythmic risk of drugs – a middle ground might be the more reasonable choice for both the FDA and the pharmaceutical industry.

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Appendix

Appendix 1.1

For a matrix with double compound symmetric structure $\boldsymbol{I}_J \otimes \boldsymbol{\Omega} + {\sigma_s}^2 * \boldsymbol{J}_{JT\times JT}$, its inverse, if exists, is $\boldsymbol{I}_J \otimes \boldsymbol{\Omega^{-1}} - \frac{{\sigma_s}^2}{(T*{\sigma_p}^2+\sigma^2)*(T*{\sigma_p}^2+\sigma^2+JT*{\sigma_s}^2)}\boldsymbol{J}_{JT\times JT}$.

Here $\boldsymbol{\Omega}= \sigma^2 * \boldsymbol{I}_T + {\sigma_p}^2 * \boldsymbol{J}_{T\times T}$.

Proof:

Let $= \boldsymbol{I}_J \otimes \boldsymbol{\Omega}$, $\boldsymbol{H} = {\sigma_s}^2 * \boldsymbol{J}_{JT\times JT}$, it's obvious that matrix $\boldsymbol{H}$ has rank one. According to Miller (1981),

$$(\boldsymbol{G}+\boldsymbol{H})^{-1} = \boldsymbol{G^{-1}} - \frac{1}{1+g}\boldsymbol{G^{-1}HG^{-1}}$$

where $g = tr(\boldsymbol{HG^{-1}})$.

According Dobbin et al. 2005,

$$\boldsymbol{\Omega^{-1}} = \frac{1}{\sigma^2} * \boldsymbol{I_T} - \frac{{\sigma_p}^2}{\sigma^2 * \left(\sigma^2 + \mathrm{T} * {\sigma_p}^2\right)} * \boldsymbol{J_T}$$

$$g = tr(\boldsymbol{HG^{-1}}) = tr\left({\sigma_s}^2 * \boldsymbol{J}_{JT\times JT} * \boldsymbol{I}_J \otimes \boldsymbol{\Omega^{-1}}\right)$$

$$= {\sigma_s}^2 * JT * \left[\frac{1}{\sigma^2} - \frac{T * {\sigma_p}^2}{\sigma^2 * \left(\sigma^2 + \mathrm{T} * {\sigma_p}^2\right)}\right]$$

$$= \frac{{\sigma_s}^2 * JT}{\left(\sigma^2 + \mathrm{T} * {\sigma_p}^2\right)}$$

$$\boldsymbol{G^{-1}HG^{-1}} = {\sigma_s}^2 * (\boldsymbol{I}_J \otimes \boldsymbol{\Omega^{-1}}) * \boldsymbol{J}_{JT\times JT} * (\boldsymbol{I}_J \otimes \boldsymbol{\Omega^{-1}})$$

$$= \frac{{\sigma_s}^2}{\left(\sigma^2 + \mathrm{T} * {\sigma_p}^2\right)} * \boldsymbol{J}_{JT\times JT} * (\boldsymbol{I}_J \otimes \boldsymbol{\Omega^{-1}}) = \frac{{\sigma_s}^2}{\left(\sigma^2 + \mathrm{T} * {\sigma_p}^2\right)^2} * \boldsymbol{J}_{JT\times JT}$$

So

$$(\boldsymbol{G}+\boldsymbol{H})^{-1} = \boldsymbol{G}^{-1} - \frac{1}{1+g}\boldsymbol{G}^{-1}\boldsymbol{H}\boldsymbol{G}^{-1}$$

$$= \boldsymbol{I}_J \otimes \boldsymbol{\Omega}^{-1} - \frac{1}{1+\frac{{\sigma_s}^2 * JT}{\left(\sigma^2 + \mathrm{T} * {\sigma_p}^2\right)}} * \frac{{\sigma_s}^2}{\left(\sigma^2 + \mathrm{T} * {\sigma_p}^2\right)^2} * \boldsymbol{J}_{JT\times JT}$$

$$= \boldsymbol{I}_J \otimes \boldsymbol{\Omega}^{-1} - \frac{{\sigma_s}^2}{\left(T * {\sigma_p}^2 + \sigma^2\right) * \left(T * {\sigma_p}^2 + \sigma^2 + JT * {\sigma_s}^2\right)}\boldsymbol{J}_{JT\times JT}$$

Appendix 1.2

For a matrix $\boldsymbol{V}_r$with block diagonal structure and each block with double compound symmetric structure $\boldsymbol{V}_r = \boldsymbol{I}_K \otimes \boldsymbol{\Sigma_r}$, where $\boldsymbol{\Sigma_r} = \boldsymbol{I}_J \otimes \boldsymbol{\Omega} + {\sigma_s}^2 * \boldsymbol{J}_{JT\times JT}$, let $\boldsymbol{E} = \mathbb{1}_{\boldsymbol{KJ}} \otimes \boldsymbol{I_T}$, then $(\boldsymbol{E}'{\boldsymbol{V}_r}^{-1}\boldsymbol{E})^{-1} = \frac{1}{K}(\frac{1}{J}\boldsymbol{\Omega} + {\sigma_s}^2\boldsymbol{J}_{T\times T})$.

Proof:

$$\boldsymbol{E}'{\boldsymbol{V}_r}^{-1}\boldsymbol{E} = [\boldsymbol{I_T}\ \boldsymbol{I_T}\ \ldots\ \boldsymbol{I_T}] * \left(\boldsymbol{I}_K \otimes {\boldsymbol{\Sigma_r}}^{-1}\right) * \begin{bmatrix}\boldsymbol{I_T}\\ \ldots \\ \boldsymbol{I_T}\end{bmatrix}$$

$$= [\boldsymbol{I_T}\ \boldsymbol{I_T}\ \ldots\ \boldsymbol{I_T}] * [\boldsymbol{I}_{KJ} \otimes \boldsymbol{\Omega}^{-1} - \frac{{\sigma_s}^2}{\left(T * {\sigma_p}^2 + \sigma^2\right) * \left(T * {\sigma_p}^2 + \sigma^2 + JT * {\sigma_s}^2\right)} * \left(\boldsymbol{I}_K \otimes \boldsymbol{J}_{JT\times JT}\right)] * \begin{bmatrix}\boldsymbol{I_T}\\ \ldots \\ \boldsymbol{I_T}\end{bmatrix}$$

$$= [{\mathbb{1}_{\boldsymbol{KJ}}}' \otimes \boldsymbol{\Omega}^{-1} - \frac{J * {\sigma_s}^2}{\left(T * {\sigma_p}^2 + \sigma^2\right) * \left(T * {\sigma_p}^2 + \sigma^2 + JT * {\sigma_s}^2\right)} * \left({\mathbb{1}_{\boldsymbol{KJ}}}' \otimes \boldsymbol{J}_{T\times T}\right)] * \begin{bmatrix}\boldsymbol{I_T}\\ \ldots \\ \boldsymbol{I_T}\end{bmatrix}$$

$$= KJ * \boldsymbol{\Omega}^{-1} - \frac{K * J^2 * {\sigma_s}^2}{\left(T * {\sigma_p}^2 + \sigma^2\right) * \left(T * {\sigma_p}^2 + \sigma^2 + JT * {\sigma_s}^2\right)} * \boldsymbol{J}_{T\times T}$$

$$= KJ * [\boldsymbol{\Omega}^{-1} - \frac{J{\sigma_s}^2}{\left(T * {\sigma_p}^2 + \sigma^2\right) * \left(T * {\sigma_p}^2 + \sigma^2 + JT * {\sigma_s}^2\right)} * \boldsymbol{J}_{T\times T}]$$

According to Appendix 1.1, we have

$$[\boldsymbol{\Omega^{-1}} - \frac{J{\sigma_s}^2}{(T*{\sigma_p}^2+\sigma^2)*(T*{\sigma_p}^2+\sigma^2+JT*{\sigma_s}^2)} * \boldsymbol{J}_{T\times T}]^{-1} = \boldsymbol{\Omega} + J{\sigma_s}^2\boldsymbol{J}_{T\times T},$$

So $(\boldsymbol{E'V_r^{-1}E})^{-1} = \frac{1}{K}(\frac{1}{J}\boldsymbol{\Omega} + {\sigma_s}^2\boldsymbol{J}_{T\times T})$.

Appendix 1.3

For rQTc model, the inverse of $var(\hat{\beta})$ is

$$\sum_{k=1}^{K}\sum_{j=1}^{J}(\boldsymbol{C}_{kj} - \overline{\boldsymbol{C}}_{..})'\boldsymbol{\Omega}^{-1}(\boldsymbol{C}_{kj} - \overline{\boldsymbol{C}}_{..}) - \frac{{\sigma_s}^2}{(T*{\sigma_p}^2+\sigma^2)*(T*{\sigma_p}^2+\sigma^2+\mathrm{JT}*{\sigma_s}^2)}(\sum_{\mathrm{k}=1}^{\mathrm{K}}{\mathrm{C_{k..}}}^2 - \frac{1}{\mathrm{K}}*{\mathrm{C_{...}}}^2).$$

Proof:

$$\frac{1}{var(\hat{\beta})} = \boldsymbol{C'V_r^{-1}C} - \boldsymbol{C'V_r^{-1}E} * (\boldsymbol{E'V_r^{-1}E})^{-1} * \boldsymbol{E'V_r^{-1}C}$$

$$\boldsymbol{V_r^{-1}E} * (\boldsymbol{E'V_r^{-1}E})^{-1}$$

$$= \left[\mathbb{1}_{KJ} \otimes \boldsymbol{\Omega^{-1}} - \frac{{\sigma_s}^2 * J}{(T*{\sigma_p}^2+\sigma^2)*(T*{\sigma_p}^2+\sigma^2+JT*{\sigma_s}^2)}\boldsymbol{J}_{KJT\times T}\right] * \frac{1}{K}\left(\frac{1}{J}\boldsymbol{\Omega} + {\sigma_s}^2\boldsymbol{J}_{T\times T}\right)$$

$$= \frac{1}{KJ}\mathbb{1}_{KJ} \otimes \boldsymbol{I_T} + \frac{{\sigma_s}^2}{K*(T*{\sigma_p}^2+\sigma^2)}\boldsymbol{J}_{KJT\times T} - \frac{{\sigma_s}^2}{K*(T*{\sigma_p}^2+\sigma^2+JT*{\sigma_s}^2)}\boldsymbol{J}_{KJT\times T}$$
$$- \frac{{\sigma_s}^4*JT}{K*(T*{\sigma_p}^2+\sigma^2)*(T*{\sigma_p}^2+\sigma^2+JT*{\sigma_s}^2)}\boldsymbol{J}_{KJT\times T}$$

$$= \frac{1}{KJ}\mathbb{1}_{KJ} \otimes \boldsymbol{I_T} + \frac{(T*{\sigma_p}^2+\sigma^2+JT*{\sigma_s}^2)*{\sigma_s}^2 - (T*{\sigma_p}^2+\sigma^2)*{\sigma_s}^2 - {\sigma_s}^4*JT}{K*(T*{\sigma_p}^2+\sigma^2)*(T*{\sigma_p}^2+\sigma^2+JT*{\sigma_s}^2)}$$

$$= \frac{1}{KJ}\mathbb{1}_{KJ} \otimes \boldsymbol{I_T} = \frac{1}{KJ}\boldsymbol{E}$$

$$\boldsymbol{V_r^{-1}E} * (\boldsymbol{E'V_r^{-1}E})^{-1} * \boldsymbol{E'V_r^{-1}}$$

$$= \frac{1}{KJ}\boldsymbol{E} * \left[\mathbb{1}'_{KJ} \otimes \boldsymbol{\Omega^{-1}} - \frac{{\sigma_s}^2 * J}{(T*{\sigma_p}^2+\sigma^2)*(T*{\sigma_p}^2+\sigma^2+JT*{\sigma_s}^2)}\boldsymbol{J}_{T\times KJT}\right]$$

$$= \frac{1}{KJ}\boldsymbol{J}_{KJ\times KJ} \otimes \boldsymbol{\Omega^{-1}} - \frac{{\sigma_s}^2}{K*(T*{\sigma_p}^2+\sigma^2)*(T*{\sigma_p}^2+\sigma^2+JT*{\sigma_s}^2)}\boldsymbol{J}_{KJT\times KJT}$$

$$\boldsymbol{C'V_r}^{-1}\boldsymbol{C} - \boldsymbol{C'V_r}^{-1}\boldsymbol{E} * (\boldsymbol{E'V_r}^{-1}\boldsymbol{E})^{-1} * \boldsymbol{E'V_r}^{-1}\boldsymbol{C}$$
$$= \boldsymbol{C'}[\boldsymbol{V_r}^{-1} - \boldsymbol{V_r}^{-1}\boldsymbol{E} * (\boldsymbol{E'V_r}^{-1}\boldsymbol{E})^{-1} * \boldsymbol{E'V_r}^{-1}]\boldsymbol{C}$$
$$= \boldsymbol{C'}\left\{\left[\boldsymbol{I}_{KJ} \otimes \boldsymbol{\Omega^{-1}} - \frac{{\sigma_s}^2}{\left(T * {\sigma_p}^2 + \sigma^2\right) * \left(T * {\sigma_p}^2 + \sigma^2 + JT * {\sigma_s}^2\right)} * \left(\boldsymbol{I}_K \otimes \boldsymbol{J}_{JT\times JT}\right)\right]\right.$$
$$- \left[\frac{1}{KJ}\boldsymbol{J}_{KJ\times KJ} \otimes \boldsymbol{\Omega^{-1}}\right.$$
$$\left.\left. - \frac{{\sigma_s}^2}{K * \left(T * {\sigma_p}^2 + \sigma^2\right) * \left(T * {\sigma_p}^2 + \sigma^2 + JT * {\sigma_s}^2\right)}\boldsymbol{J}_{KJT\times KJT}\right]\right\}\boldsymbol{C}$$
$$= \boldsymbol{C'}\left[\boldsymbol{I}_{KJ} \otimes \boldsymbol{\Omega^{-1}} - \frac{1}{KJ}\boldsymbol{J}_{KJ\times KJ} \otimes \boldsymbol{\Omega^{-1}}\right]\boldsymbol{C}$$
$$- \frac{{\sigma_s}^2}{\left(T * {\sigma_p}^2 + \sigma^2\right) * \left(T * {\sigma_p}^2 + \sigma^2 + JT * {\sigma_s}^2\right)}\boldsymbol{C'}\left[\boldsymbol{I}_K \otimes \boldsymbol{J}_{JT\times JT}\right.$$
$$\left. - \frac{1}{K}\boldsymbol{J}_{KJT\times KJT}\right]\boldsymbol{C}$$

$$\boldsymbol{C'}\left[\boldsymbol{I}_{KJ} \otimes \boldsymbol{\Omega^{-1}} - \frac{1}{KJ}\boldsymbol{J}_{KJ\times KJ} \otimes \boldsymbol{\Omega^{-1}}\right]\boldsymbol{C}$$
$$= \sum_{k=1}^{K}\sum_{j=1}^{J}\boldsymbol{C}_{kj}{}'\boldsymbol{\Omega}^{-1}\,\boldsymbol{C}_{kj} - KJ * (\overline{\boldsymbol{C}_{..}}' * \boldsymbol{\Omega}^{-1} * \overline{\boldsymbol{C}_{..}})$$
$$= \sum_{k=1}^{K}\sum_{j=1}^{J}\left(\boldsymbol{C}_{kj} - \overline{\boldsymbol{C}_{..}}\right)'\boldsymbol{\Omega}^{-1}\left(\boldsymbol{C}_{kj} - \overline{\boldsymbol{C}_{..}}\right)$$

$$\boldsymbol{C'}\left[\boldsymbol{I}_K \otimes \boldsymbol{J}_{JT\times JT} - \frac{1}{K}\boldsymbol{J}_{KJT\times KJT}\right]\boldsymbol{C}$$
$$= \boldsymbol{C'} * \left(\boldsymbol{I}_K \otimes \boldsymbol{J}_{JT\times JT}\right) * \boldsymbol{C} - \boldsymbol{C'} * \left(\frac{1}{K}\boldsymbol{J}_{KJT\times KJT}\right) * \boldsymbol{C}$$
$$= \sum_{k=1}^{K}{\mathrm{C_{k..}}}^2 - K * \overline{\mathrm{C_{...}}}^2 = \sum_{k=1}^{K}(\mathrm{C_{k..}} - \overline{\mathrm{C_{...}}})^2$$

So
$\frac{1}{var(\widehat{\beta})} = \sum_{k=1}^{K}\sum_{j=1}^{J}\left(\boldsymbol{C}_{kj} - \overline{\boldsymbol{C}_{..}}\right)'\boldsymbol{\Omega}^{-1}\left(\boldsymbol{C}_{kj} - \overline{\boldsymbol{C}_{..}}\right) - \frac{{\sigma_s}^2}{\left(T*{\sigma_p}^2+\sigma^2\right)*\left(T*{\sigma_p}^2+\sigma^2+\mathrm{JT}*{\sigma_s}^2\right)}\sum_{k=1}^{K}(\mathrm{C_{k..}} - \overline{\mathrm{C_{...}}})^2$

Appendix 1.4

$$\begin{bmatrix} \boldsymbol{E'\widetilde{V_c}^{-1}E} & \boldsymbol{E'\widetilde{V_c}^{-1}B} \\ \boldsymbol{B'\widetilde{V_c}^{-1}E} & \boldsymbol{B'\widetilde{V_c}^{-1}B} \end{bmatrix}^{-1} = \begin{bmatrix} \frac{1}{KJ}\boldsymbol{\gamma} + \overline{\boldsymbol{B}_{..}} * \boldsymbol{INV} * \overline{\boldsymbol{B}_{..}}' & -\overline{\boldsymbol{B}_{..}} * \boldsymbol{INV} \\ -\boldsymbol{INV} * \overline{\boldsymbol{B}_{..}}' & \boldsymbol{INV} \end{bmatrix}$$

where $\boldsymbol{INV} = \left(\sum_{k=1}^{K}\sum_{j=1}^{J}(\boldsymbol{B_{ij}} - \overline{\boldsymbol{B}_{..}})'\boldsymbol{\gamma^{-1}}(\boldsymbol{B_{ij}} - \overline{\boldsymbol{B}_{..}})\right)^{-1}$.

Proof:

According to equation (8) in Henderson et al (1981), when $\boldsymbol{A}$ is nonsingular and $\boldsymbol{D}$ is possibly singular

$$\begin{bmatrix} \boldsymbol{A} & \boldsymbol{U} \\ \boldsymbol{V} & \boldsymbol{D} \end{bmatrix}^{-1} = \begin{bmatrix} \boldsymbol{A^{-1}} + \boldsymbol{A^{-1}U(D - VA^{-1}U)^{-1}VA^{-1}} & -\boldsymbol{A^{-1}U(D - VA^{-1}U)^{-1}} \\ -\boldsymbol{(D - VA^{-1}U)^{-1}VA^{-1}} & \boldsymbol{(D - VA^{-1}U)^{-1}} \end{bmatrix}$$

Let $\boldsymbol{A} = \boldsymbol{E'\widetilde{V_c}^{-1}E}$, $\boldsymbol{U} = \boldsymbol{E'\widetilde{V_c}^{-1}B}$, $\boldsymbol{V} = \boldsymbol{B'\widetilde{V_c}^{-1}E}$, $\boldsymbol{D} = \boldsymbol{B'\widetilde{V_c}^{-1}B}$.

$\boldsymbol{B_{..}} = \sum_{k=1}^{K}\sum_{j=1}^{J}\boldsymbol{B_{ij}}$, $\overline{\boldsymbol{B}_{..}} = \frac{1}{KJ}\boldsymbol{B_{..}}$.

$$\boldsymbol{A^{-1}} = [\boldsymbol{E'(I_{KJ} \otimes \gamma)^{-1}E}]^{-1} = \frac{1}{KJ}\boldsymbol{\gamma}$$

$$\boldsymbol{U} = \boldsymbol{E'\widetilde{V_c}^{-1}B} = \boldsymbol{E'(I_{KJ} \otimes \gamma)^{-1}B} = \boldsymbol{\gamma^{-1}B_{..}}$$

$$\boldsymbol{V} = \boldsymbol{B'\widetilde{V_c}^{-1}E} = \boldsymbol{B'(I_{KJ} \otimes \gamma)^{-1}E} = \boldsymbol{B_{..}'\gamma^{-1}}$$

$$\boldsymbol{(D - VA^{-1}U)^{-1}} = \left(\boldsymbol{B'\widetilde{V_c}^{-1}B} - \frac{1}{KJ}\boldsymbol{B_{..}'\gamma^{-1}\gamma\gamma^{-1}B_{..}}\right)^{-1}$$

$$= \left(\sum_{k=1}^{K}\sum_{j=1}^{J}\boldsymbol{B_{ij}'\gamma^{-1}B_{ij}} - \frac{1}{KJ}\boldsymbol{B_{..}'\gamma^{-1}B_{..}}\right)^{-1}$$

$$= \left(\sum_{k=1}^{K}\sum_{j=1}^{J}(\boldsymbol{B_{ij}} - \overline{\boldsymbol{B}_{..}})'\boldsymbol{\gamma^{-1}}(\boldsymbol{B_{ij}} - \overline{\boldsymbol{B}_{..}})\right)^{-1} = \boldsymbol{INV}$$

$$\boldsymbol{A^{-1}} + \boldsymbol{A^{-1}U(D - VA^{-1}U)^{-1}VA^{-1}}$$

$$= \frac{1}{KJ}\boldsymbol{\gamma} + \frac{1}{KJ}\boldsymbol{\gamma\gamma^{-1}}\boldsymbol{B_{..}} * \boldsymbol{INV} * \boldsymbol{B'_{..}}\boldsymbol{\gamma^{-1}} * \frac{1}{KJ}\boldsymbol{\gamma} = \frac{1}{KJ}\boldsymbol{\gamma} + \overline{\boldsymbol{B}}_{\boldsymbol{..}} * \boldsymbol{INV} * \overline{\boldsymbol{B}}_{\boldsymbol{..}}'$$

$$-\boldsymbol{(D - VA^{-1}U)^{-1}VA^{-1}}$$

$$= -\boldsymbol{INV} * \boldsymbol{B'_{..}}\boldsymbol{\gamma^{-1}} * \frac{1}{KJ}\boldsymbol{\gamma} = -\boldsymbol{INV} * \overline{\boldsymbol{B}}_{\boldsymbol{..}}'$$

$$-\boldsymbol{A^{-1}U(D - VA^{-1}U)^{-1}}$$

$$= -\frac{1}{KJ}\boldsymbol{\gamma}\boldsymbol{B_{..}} * \boldsymbol{INV} = -\overline{\boldsymbol{B}}_{\boldsymbol{..}} * \boldsymbol{INV}$$

So

$$\begin{bmatrix} \boldsymbol{E'}\widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}}\boldsymbol{E} & \boldsymbol{E'}\widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}}\boldsymbol{B} \\ \boldsymbol{B'}\widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}}\boldsymbol{E} & \boldsymbol{B'}\widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}}\boldsymbol{B} \end{bmatrix}^{-1} = \begin{bmatrix} \frac{1}{KJ}\boldsymbol{\gamma} + \overline{\boldsymbol{B}}_{\boldsymbol{..}} * \boldsymbol{INV} * \overline{\boldsymbol{B}}_{\boldsymbol{..}}' & -\overline{\boldsymbol{B}}_{\boldsymbol{..}} * \boldsymbol{INV} \\ -\boldsymbol{INV} * \overline{\boldsymbol{B}}_{\boldsymbol{..}}' & \boldsymbol{INV} \end{bmatrix}$$

where $\boldsymbol{INV} = \left(\sum_{k=1}^{K}\sum_{j=1}^{J}(\boldsymbol{B_{ij}} - \overline{\boldsymbol{B}}_{\boldsymbol{..}})'\boldsymbol{\gamma^{-1}}(\boldsymbol{B_{ij}} - \overline{\boldsymbol{B}}_{\boldsymbol{..}})\right)^{\boldsymbol{-1}}$.

Appendix 1.5

$$\widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}}\boldsymbol{E_1} * (\boldsymbol{E_1'}\widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}}\boldsymbol{E_1})^{\boldsymbol{-1}} * \boldsymbol{E_1}'\widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}}$$

$$= \frac{1}{KJ}\boldsymbol{J_{KJ\times KJ}} \otimes \boldsymbol{\gamma^{-1}} + \widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}}(\boldsymbol{B} - \boldsymbol{E} * \overline{\boldsymbol{B}}_{\boldsymbol{..}}) * \left[(\boldsymbol{B} - \boldsymbol{E} * \overline{\boldsymbol{B}}_{\boldsymbol{..}})'\widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}}(\boldsymbol{B} - \boldsymbol{E} * \overline{\boldsymbol{B}}_{\boldsymbol{..}})\right]^{\boldsymbol{-1}}$$
$$* (\boldsymbol{B} - \boldsymbol{E} * \overline{\boldsymbol{B}}_{\boldsymbol{..}})'\widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}}$$

Proof:

$$\widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}}\boldsymbol{E_1} = \widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}}[\boldsymbol{E} \quad \boldsymbol{B}] = [\widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}}\boldsymbol{E} \quad \widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}}\boldsymbol{B}]$$

$$\boldsymbol{E_1'}\widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}} = \begin{bmatrix} \boldsymbol{E'}\widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}} \\ \boldsymbol{B'}\widetilde{\boldsymbol{V_c}}^{\boldsymbol{-1}} \end{bmatrix}$$

$$\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E_1} * \left(\boldsymbol{E_1'}\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E_1}\right)^{-1} * \boldsymbol{E_1'}\widetilde{\boldsymbol{V_c}}^{-1}$$

$$= \left[\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E} \quad \widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{B}\right] * \begin{bmatrix} \frac{1}{KJ}\boldsymbol{\gamma} + \overline{\boldsymbol{B}}_{\cdot\cdot} * \boldsymbol{INV} * \overline{\boldsymbol{B}}_{\cdot\cdot}' & -\overline{\boldsymbol{B}}_{\cdot\cdot} * \boldsymbol{INV} \\ -\boldsymbol{INV} * \overline{\boldsymbol{B}}_{\cdot\cdot}' & \boldsymbol{INV} \end{bmatrix} * \begin{bmatrix} \boldsymbol{E'}\widetilde{\boldsymbol{V_c}}^{-1} \\ \boldsymbol{B'}\widetilde{\boldsymbol{V_c}}^{-1} \end{bmatrix}$$

$$= \left[\frac{1}{KJ}\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E} * \boldsymbol{\gamma} + \widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E} * \overline{\boldsymbol{B}}_{\cdot\cdot} * \boldsymbol{INV} * \overline{\boldsymbol{B}}_{\cdot\cdot}' - \widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{B} * \boldsymbol{INV} * \overline{\boldsymbol{B}}_{\cdot\cdot}' \quad - \widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E} * \overline{\boldsymbol{B}}_{\cdot\cdot} * \boldsymbol{INV} \right.$$
$$\left. + \widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{B} * \boldsymbol{INV}\right] * \begin{bmatrix} \boldsymbol{E'}\widetilde{\boldsymbol{V_c}}^{-1} \\ \boldsymbol{B'}\widetilde{\boldsymbol{V_c}}^{-1} \end{bmatrix}$$

$$= \frac{1}{KJ}\widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E} * \boldsymbol{\gamma} * \boldsymbol{E'}\widetilde{\boldsymbol{V_c}}^{-1} + \widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E} * \overline{\boldsymbol{B}}_{\cdot\cdot} * \boldsymbol{INV} * \overline{\boldsymbol{B}}_{\cdot\cdot}' * \boldsymbol{E'}\widetilde{\boldsymbol{V_c}}^{-1} - \widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{B} * \boldsymbol{INV} * \overline{\boldsymbol{B}}_{\cdot\cdot}' * \boldsymbol{E'}\widetilde{\boldsymbol{V_c}}^{-1}$$
$$- \widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E} * \overline{\boldsymbol{B}}_{\cdot\cdot} * \boldsymbol{INV} * \boldsymbol{B'}\widetilde{\boldsymbol{V_c}}^{-1} + \widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{B} * \boldsymbol{INV} * \boldsymbol{B'}\widetilde{\boldsymbol{V_c}}^{-1}$$

$$= \frac{1}{KJ}\boldsymbol{J_{KJ\times KJ}} \otimes \boldsymbol{\gamma^{-1}} + \widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{E} * \overline{\boldsymbol{B}}_{\cdot\cdot} * \boldsymbol{INV} * \left(\overline{\boldsymbol{B}}_{\cdot\cdot}' * \boldsymbol{E'}\widetilde{\boldsymbol{V_c}}^{-1} - \boldsymbol{B'}\widetilde{\boldsymbol{V_c}}^{-1}\right) + \widetilde{\boldsymbol{V_c}}^{-1}\boldsymbol{B} * \boldsymbol{INV} * (\boldsymbol{B'}\widetilde{\boldsymbol{V_c}}^{-1}$$
$$- \overline{\boldsymbol{B}}_{\cdot\cdot}' * \boldsymbol{E'}\widetilde{\boldsymbol{V_c}}^{-1})$$

$$= \frac{1}{KJ}\boldsymbol{J_{KJ\times KJ}} \otimes \boldsymbol{\gamma^{-1}} + \widetilde{\boldsymbol{V_c}}^{-1}(\boldsymbol{B} - \boldsymbol{E} * \overline{\boldsymbol{B}}_{\cdot\cdot}) * \boldsymbol{INV} * (\boldsymbol{B} - \boldsymbol{E} * \overline{\boldsymbol{B}}_{\cdot\cdot})'\widetilde{\boldsymbol{V_c}}^{-1}$$

$$= \frac{1}{KJ}\boldsymbol{J_{KJ\times KJ}} \otimes \boldsymbol{\gamma^{-1}} + \widetilde{\boldsymbol{V_c}}^{-1}(\boldsymbol{B} - \boldsymbol{E} * \overline{\boldsymbol{B}}_{\cdot\cdot}) * \left[(\boldsymbol{B} - \boldsymbol{E} * \overline{\boldsymbol{B}}_{\cdot\cdot})'\widetilde{\boldsymbol{V_c}}^{-1}(\boldsymbol{B} - \boldsymbol{E} * \overline{\boldsymbol{B}}_{\cdot\cdot})\right]^{-1}$$
$$* (\boldsymbol{B} - \boldsymbol{E} * \overline{\boldsymbol{B}}_{\cdot\cdot})'\widetilde{\boldsymbol{V_c}}^{-1}$$

Appendix 1.6

(3.4.1) − (3.4.2)

$$= \frac{1}{T * {\sigma_p}^2 + \sigma^2}\left[\frac{\sigma^2 - {\sigma_p}^2}{\sigma^2(T+1)}\sum_{k=1}^{K}\sum_{j=1}^{J}\left(C_{kj\cdot} - \frac{C_{\cdots}}{KJ}\right)^2 - \frac{{\sigma_s}^2}{\left(T * {\sigma_p}^2 + \sigma^2 + \mathrm{JT} * {\sigma_s}^2\right)}\sum_{k=1}^{K}\left(C_{k\cdot\cdot} - \frac{C_{\cdots}}{K}\right)^2\right]$$

Proof:

(3.4.1)=

$$\sum_{k=1}^{K}\sum_{j=1}^{J}\left(\boldsymbol{C}_{kj}-\overline{\boldsymbol{C}_{..}}\right)'\boldsymbol{\Omega}^{-1}\left(\boldsymbol{C}_{kj}-\overline{\boldsymbol{C}_{..}}\right)$$

$$-\frac{{\sigma_s}^2}{\left(T*{\sigma_p}^2+\sigma^2\right)*\left(T*{\sigma_p}^2+\sigma^2+\mathrm{JT}*{\sigma_s}^2\right)}\sum_{k=1}^{K}(\mathrm{C_{k..}}-\overline{\mathrm{C_{...}}})^2$$

(3.4.2)=

$$\sum_{k=1}^{K}\sum_{j=1}^{J}\left(\boldsymbol{C}_{kj}-\overline{\boldsymbol{C}_{..}}\right)'\boldsymbol{\Gamma}^{-1}\left(\boldsymbol{C}_{kj}-\overline{\boldsymbol{C}_{..}}\right)$$

$$\boldsymbol{\Omega^{-1}-\Gamma^{-1}}=\left(\frac{1}{\sigma^2}*\boldsymbol{I}_T-\frac{{\sigma_p}^2}{\left(T{\sigma_p}^2+\sigma^2\right)*\sigma^2}*\boldsymbol{J}_T\right)-\left(\frac{1}{\sigma^2}*\boldsymbol{I}_T-\frac{1}{(T+1)\sigma^2}*\boldsymbol{J}_T\right)$$

$$=\frac{\sigma^2-{\sigma_p}^2}{(T+1)\sigma^2\left(T{\sigma_p}^2+\sigma^2\right)}*\boldsymbol{J}_T$$

$(3.4.1)-(3.4.2)$

$$=\sum_{k=1}^{K}\sum_{j=1}^{J}\left(\boldsymbol{C}_{kj}-\overline{\boldsymbol{C}_{..}}\right)'\left(\boldsymbol{\Omega}^{-1}-\boldsymbol{\Gamma}^{-1}\right)\left(\boldsymbol{C}_{kj}-\overline{\boldsymbol{C}_{..}}\right)$$

$$-\frac{{\sigma_s}^2}{\left(T*{\sigma_p}^2+\sigma^2\right)*\left(T*{\sigma_p}^2+\sigma^2+\mathrm{JT}*{\sigma_s}^2\right)}\left(\sum_{k=1}^{K}{C_{k..}}^2-\frac{1}{K}*{C_{...}}^2\right)$$

$$=\frac{\sigma^2-{\sigma_p}^2}{\sigma^2(T+1)*\left(T*{\sigma_p}^2+\sigma^2\right)}\sum_{k=1}^{K}\sum_{\boldsymbol{j=1}}^{\boldsymbol{J}}\left(\boldsymbol{C_{kj}}-\overline{\boldsymbol{C}_{..}}\right)'\boldsymbol{J_{T\times T}}\left(\boldsymbol{C_{kj}}-\overline{\boldsymbol{C}_{..}}\right)$$

$$-\frac{{\sigma_s}^2}{\left(T*{\sigma_p}^2+\sigma^2\right)*\left(T*{\sigma_p}^2+\sigma^2+\mathrm{JT}*{\sigma_s}^2\right)}\left(\sum_{k=1}^{K}{C_{k..}}^2-\frac{1}{K}*{C_{...}}^2\right)$$

$$=\frac{\sigma^2-{\sigma_p}^2}{\sigma^2(T+1)*\left(T*{\sigma_p}^2+\sigma^2\right)}\sum_{k=1}^{K}\sum_{j=1}^{J}\left(C_{kj.}-\frac{C_{...}}{KJ}\right)^2$$

$$-\frac{{\sigma_s}^2}{\left(T*{\sigma_p}^2+\sigma^2\right)*\left(T*{\sigma_p}^2+\sigma^2+\mathrm{JT}*{\sigma_s}^2\right)}\sum_{k=1}^{K}\left(C_{k..}-\frac{C_{...}}{K}\right)^2$$

$$= \frac{1}{T * {\sigma_p}^2 + \sigma^2} \left[\frac{\sigma^2 - {\sigma_p}^2}{\sigma^2 (T+1)} \sum_{k=1}^{K} \sum_{j=1}^{J} \left(C_{kj.} - \frac{C_{...}}{KJ} \right)^2 - \frac{{\sigma_s}^2}{\left(T * {\sigma_p}^2 + \sigma^2 + \mathrm{JT} * {\sigma_s}^2 \right)} \sum_{k=1}^{K} \left(C_{k..} - \frac{C_{...}}{K} \right)^2 \right]$$